frontiers | Research Topics

Origin, conservation, and restoration of the threatened European grassland ecosystem in the Anthropocene

Edited by
Grégory Mahy, Thierry Dutoit, Kathrin Kiehl,
Armin Bischoff and Péter Török

Published in
Frontiers in Ecology and Evolution
Frontiers in Environmental Science

ISSN 1664-8714
ISBN 978-2-8325-4406-8
DOI 10.3389/978-2-8325-4406-8

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Origin, conservation, and restoration of the threatened European grassland ecosystem in the Anthropocene

Topic editors
Grégory Mahy — University of Liège, Belgium
Thierry Dutoit — UMR7263 Institut méditerranéen de biodiversité et d'écologie marine et continentale (IMBE), France
Kathrin Kiehl — Osnabrück University of Applied Sciences, Germany
Armin Bischoff — University of Avignon, France
Péter Török — University of Debrecen, Hungary

Citation
Mahy, G., Dutoit, T., Kiehl, K., Bischoff, A., Török, P., eds. (2024). *Origin, conservation, and restoration of the threatened European grassland ecosystem in the Anthropocene*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-4406-8

Table of contents

frontiers | Frontiers in Ecology and Evolution

TYPE Editorial
PUBLISHED 30 October 2024
DOI 10.3389/fevo.2024.1487211

Editorial: Origin, conservation, and restoration of the threatened European grassland ecosystem in the Anthropocene

Grégory Mahy [1,2*], Peter Torok [3], Armin Bischoff [1], Kathrin Kiehl [4] and Thierry Dutoit [1*]

[1]Mediterranean Institute of Marine and Terrestrial Biodiversity and Ecology (IMBE), Aix Marseille University, National Centre for Scientific Research (CNRS), French National Research Institute for Sustainable Development (IRD), Avignon University, Avignon, France, [2]Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, [3]Department of Ecology, University of Debrecen, Debrecen, Hungary, [4]Vegetation Ecology and Botany, Osnabrück University of Applied Sciences, Osnabrück, Germany

KEYWORDS

grassland, meadow, steppe, conservation, restoration, Anthropocene, biodiversity

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Fernanda Michalski,
Universidade Federal do Amapá, Brazil

*CORRESPONDENCE
Grégory Mahy
✉ g.mahy@uliege.be
Thierry Dutoit
✉ thierry.dutoit@univ-avignon.fr

RECEIVED 27 August 2024
ACCEPTED 30 August 2024
PUBLISHED 30 October 2024

CITATION
Mahy G, Torok P, Bischoff A, Kiehl K and Dutoit T (2024) Editorial: Origin, conservation, and restoration of the threatened European grassland
ecosystem in the Anthropocene.
Front. Ecol. Evol. 12:1487211.
doi: 10.3389/fevo.2024.1487211

Editorial on the Research Topic
Origin, conservation, and restoration of the threatened European grassland ecosystem in the Anthropocene

The European continent boasts a rich tapestry of natural and semi-natural grasslands, formed through intricate interactions between humans and nature, representing not only biodiversity hotspots but also invaluable cultural heritage (Poschlod and WallisDeVries, 2002). While based on a recent estimate, Europe harbors more than 650 thousand square kilometer of permanent grasslands, on average only up to a half of this area is covered by grasslands of high natural value (HNV) (Dengler et al., 2020). Beside of the area loss and fragmentation, both intensification and abandonment of traditional extensive land use, as well as the steadily increasing urbanization threaten the species composition and diversity of these ecosystems (Habel et al., 2013; Dengler et al., 2014). As we navigate the complexities of the Anthropocene, these ecosystems face unprecedented impacts, compelling us to challenge our historical approaches to conservation and restoration. In recent years, scientific research has delved deep into understanding the diverse array of European grasslands. From some intact large and continuous grassland stands (e.g. some steppes in Eastern Europe), to patches nested within urban sprawls and the emergent green roofs adorning urban landscapes, researchers have unraveled the unique ecological dynamics and management challenges associated with each type of grassland.

With the Research Topic “Origin, Conservation, and Restoration of the Threatened European Grassland Ecosystem in the Anthropocene”, we set the stage for a journey through the nuanced landscapes of European grasslands, offering an overview of recent scientific endeavors and a call to action for the preservation and restoration of these unique ecosystems, as well as the integration of urban and novel grassland ecosystems in a global conservation strategy. The findings of the 14 studies presented in this Research Topic underline the importance of recognizing the heterogeneity within European grasslands. Whether it is the biodiversity-rich semi-natural grasslands, spawning mediterranean and temperate dry grasslands (Martin et al., Ewert et al., Blight et al., Köhler et al., Jaunatre et al.), floodplain and hay meadows (Sommer et al., Dellicour et al.), montane grasslands (Helbing et al., Durbecq et al.), loess and sand steppe fragments of the Pannonian forest steppe region (Szitár et al.), the intricate dynamics of urban green spaces (Fernandes et al., Gros et al.) or the emergent novel ecosystems such as green roofs or solar parks (Lambert et al., Rivière et al.), those ecosystems present both similar and own sets of challenges and opportunities for conservation, management and restoration. Preservation of large fragments, targeted management, and innovative restoration techniques offer pathways towards safeguarding the ecological integrity of natural and semi-natural grasslands.

Landscape complexity and configuration are key factors for the conservation of grassland biodiversity. Grassland fragments, in loess steppes and sand steppe fragments, exhibit diverse vegetation compositions influenced by the size of fragment and landscape connectivity (Szitár et al.). Both types of grasslands rely on the persistence of clonal perennial specialist and generalist species in small and isolated patches to mitigate fragmentation effects. These results are well in agreement with Deák et al. (2018), who found that generalist species were not affected but specialist species with a good dispersal strategy were the most affected by isolation via the lack of appropriate dispersal vectors in the landscape. Ewert et al. demonstrate for the first time the importance of European dry grassland fragments for bats under certain local and landscape compositional conditions in a heterogeneous agricultural region of Germany. By underlining the value of structurally heterogeneous dry grassland fragments acting as potential stepping-stones in intensively used farmland areas, they challenge the current management goals of dry grasslands, aiming to reduce shrub and tree encroachment. Besides, their results also highlight the necessity of connectivity networks in agricultural landscapes. To go further in the study of connectivity, Blight et al. explore how recent technological advances in movement ecology (passive RFID tags), make it possible to track the individual movements of three small ground-dwelling beetle species in a Mediterranean dry grassland. While the study highlight limitations of using passive tags to track the movements of small terrestrial beetles, it helps predicting the ability of species to recolonize degraded areas, enabling appropriate restoration actions to be designed based on landscape ecology principles.

Livestock grazing, that is used in conservation practice to mimic natural grazing regimes provided by wild large herbivores is also recognized as a major management driver of the composition of plant communities in natural and semi-natural grasslands. So far, very few studies have analyzed its impact on micro-scale patterns of biodiversity and vegetation dynamics. Martin et al. present the first overview of the multiple effects of grazing and soil characteristics on plant communities of micro-patterns at the micro-local scale in Mediterranean dry grasslands. They demonstrate that there is no one-size-fits-all strategy to influencing the trajectory of the plant communities and one of the main levers of restoration strategies, changing grazing pressure, may have contrasting effects depending on the community type. Moreover, Köhler et al. indicate how megaherbivore grazing, that aims to substitute extinct wild grazers, should be considered for restoration of temperate dry grassland. Year-round horse grazing as a relatively new grassland restoration tool has the potential to enhance floristic biodiversity of dry calcareous grasslands not only in the short, but also in the long term. Finally, Durbecq et al. demonstrate that, in mountain grasslands of the French Alps, initial grazing exclusion is not necessary for restoration success under current livestock density.

Restoration of European natural and semi-natural grasslands is a major challenge and face multiple questions relative to the diversity of techniques and to success monitoring. Sommer et al. show that long-term monitoring of restoration projects is necessary, as factors determining restoration success may only become evident in the long term. When restoring floodplain meadows with transfer of seed-containing plant material from species-rich donor sites, a widely used method to restore semi-natural grasslands, the positive effect of soil preparation on the number and cover of target species, which is regularly reported in short-term studies, diminishes over time, while the effects of local site conditions become more important. The importance of long-term monitoring is also confirmed in dry grassland where Köhler et al. reveal that short-term evaluation of restoration schemes can be misleading, particularly at slow-changing indicators such as dry calcareous vegetation types and slow-growing and long-lived species or those exhibiting high interannual population fluctuations such as many orchid species. Not only monitoring but also definitions of adequate restoration targets is important to promote restoration of grasslands in a changing world. Dellicour et al. show how restoration of hay meadows faces challenges in defining relevant reference ecosystems and adapting restoration techniques for the present-day ecological and socio-economic context.

Natural and semi-natural grassland restoration success is also under the control of a diversity of socio-environmental factors and requires fine-tuned methods adapted to the socio-economic context. Restoration efforts for degraded temperate lowland hay meadows show varying success rates, with techniques such as passive restoration mowing, active fresh hay transfer, and sowing of threshing material being implemented based on the initial level of degradation and proximity to well-preserved meadows (Dellicour et al.). Either abiotic, biotic and dispersal filters drive the limitation to recovery in Mediterranean dry grassland more than 40 years after an intermediate period of arable use (Jaunatre et al.). In afforested landscapes, tree removal, hay transfer and resumption of grazing or mowing are typical measures to re-create species-rich grasslands. The application of these restoration measures is effective in re-establishing target communities of different taxa (vascular plants and three insect taxa (leafhoppers, true bugs, and grasshoppers)) for German montane grasslands (Helbing et al.). Durbecq et al. highlight also the complex interactions among seed sowing, grazing regimes and soil disturbance on restoration success of mountain grasslands in the Southern French Alps. A strong grazing-by-soil disturbance interaction show that a combination of both is the best strategy to restore this grassland type.

In urban environment, landscape configuration, targeted management, and innovative restoration techniques are also key drivers for enhancing biodiversity of urban grassland. Gros et al. illustrate the importance of considering factors that act at both local and landscape scales when identifying the drivers of plant communities in urban grasslands in Western France. Mowing practices and landscape composition do not affect the richness or diversity of plant species but significantly influence the composition of communities. Landscape composition favors plant species according to their strategies, preferential habitats, and life spans. Furthermore, diversification of management practices limits the establishment of non-native species and induces a wider range of functional strategies. Moreover, the application of seed mixes with regional native species allows the promotion of native flora in Mediterranean peri-urban green areas, enhancing the potential of these spaces as tools for biodiversity conservation and diminishing the risk of introducing exotic species with invasive ability (Fernandes et al.). In the Mediterranean context and given the small number of native seed mixes tested, this study represents an innovation and provides practical insights into grassland rehabilitation, contributing to the improvement of plant diversity management in peri-urban and urban Mediterranean areas.

Novel opportunities for grasslands such as green roofs and novel land uses such as solar parks, also offer opportunities to strengthen the ecological network for grassland biodiversity while not replacing historical natural and semi natural grasslands. Lambert et al. explore how the increasing construction of solar parks may provide an opportunity to restore or even create semi-natural grasslands. Solar parks allow the establishment of semi-natural grassland communities, but solar panels partially disintegrate the relationship between climate and plant species composition. Ecosystem services provided by soil organisms such as carbon storage, nutrient regulation, and soil conservation, are thus hampered by solar panels. The ecological integration of solar parks to favor the establishment of semi-natural grasslands needs to limit the effect of panels on plant communities and soil quality. In an urban area, Riviere et al. explore how extensive green roofs support native biodiversity analogous to natural or semi-natural habitats like grasslands, highlighting their potential for urban biodiversity enhancement. The study shows the importance of seed banks in the dynamics of green roof vegetation and demonstrates that native species on green roofs exhibit similar behavior as in their natural environment.

The Research Topic demonstrates that it is imperative to learn the lessons obtained from scientific studies as we stand at the crossroads of grasslands conservation and restoration in the Anthropocene. By embracing the complexity and diversity of European grasslands and drawing upon the wealth of knowledge generated by recent research, we can chart a course towards a more sustainable coexistence with nature. Through concerted efforts, informed decision-making, and a shared commitment to stewardship, we can ensure the resilience and vitality of European grasslands for future generations.

Author contributions

GM: Conceptualization, Writing – original draft, Writing – review & editing. PT: Conceptualization, Validation, Writing – review & editing. AB: Conceptualization, Validation, Writing – review & editing. KK: Validation, Writing – review & editing. TD: Conceptualization, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. PT was supported by the National Research, Development and Innovation Office during the manuscript preparation [NKFIH KKP 144068 and K 137573]. GM was supported by FNRS (Fonds National de la Recherche Scientifique) for a sabbatical mission during the coordination of the Research Topic.

Acknowledgments

The guest editors are thankful for the authors of the Research Topic for contributing with their research to this initiative.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Deák, B., Valkó, O., Török, P., Kelemen, A., Bede, A., Csathó, A. I., et al. (2018). Landscape and habitat filters jointly drive richness and abundance of specialist plants in terrestrial habitat islands. *Landscape Ecol.* 33, 1117–1132. doi: 10.1007/s10980-018-0660-x

Dengler, J., Biurrun, I., Boch, S., Dembicz, I., and Török, P. (2020). "Grasslands of the palaearctic biogeographic realm: introduction and synthesis," in *Encyclopedia of the World's Biomes*, vol. Volume 3. (Elsevier Inc.). doi: 10.1016/B978-0-12-409548-9.12432-7

Dengler, J., Janišová, M., Török, P., and Wellstein, C. (2014). Biodiversity of Palaearctic grasslands: a synthesis. *Agric. Ecosyst. Environ.* 182, 1–14. doi: 10.1016/j.agee.2013.12.015

Habel, J. C., Dengler, J., Janišová, M., Torok, P., Wellstein, C., and Wiezig, M. P. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodivers. Conserv.* 22, 2131–2138. doi: 10.1007/s10531-013-0537-x

Poschlod, P., and WallisDeVries, M. F. (2002). The historical and socioeconomic perspective of calcareous grasslands—lessons from the distant and recent past. *Biological Conservation*. 104 (3), 361–376.

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 03 November 2022
DOI 10.3389/fevo.2022.879060

EDITED BY
Orsolya Valkó,
Hungarian Academy of Sciences, Hungary

REVIEWED BY
Antonio Greco,
University of Salento,
Italy
Gonzalo García-Baquero,
University of the Basque Country, Spain

*CORRESPONDENCE
Gabrielle Martin
gabrielle.martin@univ-tlse3.fr

SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 18 February 2022
ACCEPTED 07 October 2022
PUBLISHED 03 November 2022

CITATION
Martin G, Courtial A, Génin A, Ramone H and Dutoit T (2022) Why grazing and soil matter for dry grassland diversity: New insights from multigroup structural equation modeling of micro-patterns. *Front. Ecol. Evol.* 10:879060. doi: 10.3389/fevo.2022.879060

Why grazing and soil matter for dry grassland diversity: New insights from multigroup structural equation modeling of micro-patterns

Gabrielle Martin[1,2,3,4]*, Anouk Courtial[2], Alexandre Génin[5,6], Hervé Ramone[2] and Thierry Dutoit[2]

[1]Laboratoire EDB Évolution and Diversité Biologique UMR 5174, Université Toulouse 3 Paul Sabatier, UPS, CNRS, IRD, Université de Toulouse, Toulouse, France, [2]Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Univ Avignon, Aix Marseille Univ, CNRS, IRD, IUT site Agroparc, Avignon, France, [3]Centre d'Ecologie et des Sciences de la Conservation (CESCO), Muséum national d'Histoire naturelle, Centre National de la Recherche Scientifique, Sorbonne Université, UMR7204 Centre des Sciences de la Conservation (CESCO), Paris, Île-de-France, France, [4]Laboratoire Cogitamus, Paris, Languedoc-Roussillon, France, [5]Université de Montpellier, CNRS, EPHE, IRD, UMR5554 Institut des Sciences de l'Evolution de Montpellier (ISEM), Montpellier, France, [6]Copernicus Institute of Sustainable Development, Utrecht University, Utrecht, Netherlands

Grazing is recognized as a major process driving the composition of plant communities in grasslands, mostly due to the heterogeneous removal of plant species and soil compaction that results in a mosaic of small patches called micro-patterns. To date, no study has investigated the differences in composition and functioning among these micro-patterns in grasslands in relation to grazing and soil environmental variables at the micro-local scale. In this study, we ask (1) To what extent are micro-patterns different from each other in terms of species composition, species richness, vegetation volume, evenness, and functioning? and (2) based on multigroup structural equation modeling, are those differences directly or indirectly driven by grazing and soil characteristics? We focused on three micro-patterns of the Mediterranean dry grassland of the Crau area, a protected area traditionally grazed in the South-East of France. From 70 plant community relevés carried out in three micro-patterns located in four sites with different soil and grazing characteristics, we performed univariate, multivariate analyses and applied structural equation modeling for the first time to this type of data. Our results show evidence of clear differences among micro-pattern patches in terms of species composition, vegetation volume, species richness, evenness, and functioning at the micro-local scale. These differences are maintained not only by direct and indirect effects of grazing but also by several soil variables such as fine granulometry. Biological crusts appeared mostly driven by these soil variables, whereas reference and edge communities are mostly the result of different levels of grazing pressure revealing three distinct functioning specific to each micro-pattern, all of them coexisting at the micro-local scale in the studied Mediterranean dry grassland. This first overview of the multiple effects of grazing and soil characteristics on communities in micro-patterns is discussed within the scope of the conservation of dry grasslands plant diversity.

KEYWORDS

patchiness, soil nutrients and granulometry, grazing intensity, plant community composition, plant diversity, community functioning, micro-local scale, biological conservation

Introduction

Grasslands have been defined as ecosystems dominated by graminoids (i.e., grasses and grass-like plants) and forbs (Jacobs et al., 1999; Gibson, 2009). They constitute an important ecosystem type comprising about one-third of the Earth's vegetative cover (Jacobs et al., 1999; Suttie et al., 2005) and covering over 900,000 km^2 in Europe (Eurostat, 2017). Typical temperate grasslands are almost continuous ecosystems of grasses, sedges, and herbaceous dicotyledonous species in varying amounts, with scattered bushes and low tree cover (Jacobs et al., 1999). Their characteristics vary with local climate (i.e., water, wind, and light), soil, and topography (i.e., edaphic factors), biotic interactions but also natural and human disturbances such as fires and grazing (Burke et al., 1998; Jacobs et al., 1999; White et al., 2000; Gibson, 2009; Dantas et al., 2016).

Grazing is known to be a major driver of the composition of plant communities in grasslands, especially at the micro-local scale where grazing increases spatial heterogeneity and plant diversity relative to ungrazed habitat (McNaughton, 1983; Milchunas and Lauenroth, 1993; Adler et al., 2001; Ge and Liu, 2022). Grazing leads to the heterogeneous removal of plant species due to differences in plant palatabilities and animal selectivity (Bakker et al., 1983; Ge and Liu, 2022). These can yield a mosaic-like structure in vegetation, called micro-patterns (Bakker et al., 1983), where patches of tall vegetation coexist with shorter patches containing more palatable plants. This direct effect may be reinforced by indirect effects of grazers: for example, because grazers may forage more in patches with shorter plants, such patches can be denser, which further reduces plant development (Chenot et al., 2017). Both such direct and indirect effects combine with possible pre-existing spatial variations in soil characteristics to produce heterogeneity in vegetation composition, structure, and local functioning. Nevertheless, to our knowledge, no study has sought to disentangle so far the double effect of grazing and soil conditions on micro-pattern species composition and diversity (but see Bakker et al., 1983), especially to highlight the specific functioning at work due to grazing pressure and soil characteristics at a micro-local scale.

Yet, understanding these related effects on the species composition, abundance, and functioning of grassland communities remains crucial for the implementation of effective management strategies, restoration and conservation programs for species-rich grasslands. Indeed, some old-growth grasslands are composed of plant communities that require centuries or millennia to assemble (Henry et al., 2010; Poschlod and Baumann, 2010; Robin et al., 2018; Saatkamp et al., 2021) and exhibit an exceptional biodiversity both in terms of high plant species richness and endemism (Veldman et al., 2015). One example is the 26,000 km^2 of dry grasslands of the Northwestern Mediterranean Basin (Buisson et al., 2020). These grasslands are typically resilient to endogenous disturbance regimes such as wildfires (Bond and Keeley, 2005; Vidaller et al., 2019) or grazing pressure variability (Saatkamp et al., 2017), but extremely vulnerable to human-caused exogenous perturbations, for example those arising from cultivation (Römermann et al., 2005; Coiffait-Gombault et al., 2012; Helm et al., 2019). They are also currently threatened by an increase in farmland abandonment leading to shrub encroachment, especially in the Mediterranean area where the decrease in livestock is considered a major cause of changes in landscapes (Buisson et al., 2020). Therefore, initiatives to conserve and restore old-growth Mediterranean grasslands are urgently needed based on our understanding of processes at work in maintaining their plant diversity and functions (Veldman et al., 2015).

To shed new light on the processes that maintain the heterogeneity and plant diversity in dry grasslands, we conducted our study at the micro-local scale, focusing on three micro-pattern plant communities of the Crau nature reserve, a protected Mediterranean dry grassland located in the South-East of France. This area is the only French Mediterranean pseudo-steppe, whose origin and sustainability arise from the combination action of climate, specific soil conditions, and the traditional practice of sheep grazing for several millennia (Buisson and Dutoit, 2006). At this micro-local scale, we addressed the following questions: (1) To what extent are the plant communities of the micro-patterns different from each other in terms of species composition, species richness, vegetation volume, evenness, and functioning? and (2) are those differences directly and/or indirectly driven by grazing and/or soil variables? In order to identify the processes maintaining heterogeneity in space, and potentially affecting the temporal dynamics of these micro-patterns, we performed univariate, multivariate analyses and applied structural equation modeling for the first time to this type of data to reveal direct and indirect effects of explanatory variables. We discussed our results within the scope of plant community ecology and applications to the biological conservation of species-rich Mediterranean dry grasslands.

Materials and methods

Study area and sites

Vegetation data were collected in the Crau nature reserve, hereafter called Crau area (Figure 1). This area is characterized by a Mediterranean climate (dry summers, mild winters, with rainfall peaking in autumn, some detailed temperature and precipitation data are available for the 1997–2006 period in Wolff et al., 2015), frequent strong northwesterly winds (Saatkamp et al., 2021) and is dominated by "pseudo-steppic" vegetation (Tatin et al., 2013). Dominant species composition is characteristic of Mediterranean *Thero-Brachypodietea* habitat "Pseudosteppe with grasses and annuals," a high priority habitat for conservation (Corine Biotopes Code 34.512, Bissardon et al., 1997; Natura 2000 habitat type 6,220, San Miguel, 2008; EUNIS habitat E1.311 and E1.312, Louvel et al., 2013). This pseudo-steppic vegetation located in the fossil delta of the Durance river is characterized by a high stone cover (mainly pebbles) that limits the installation and the development of dense grasslands. Plant communities are highly diverse and dominated by low or very short herbaceous vegetation (49.4% of all plant species are annuals, Devaux et al., 1983; Helm et al., 2019; Pfeiffer et al., 2019), as a result of the combined effects of climate, soil, fire, and grazing. Grazing by itinerant sheep flocks has been reported since at least 2000 BP on the basis of Roman archeological remains (Badan et al., 1995; Henry et al., 2010; Robin et al., 2018; Saatkamp et al., 2021). This extensive grazing, occurring mostly from February to June, is heterogeneous throughout the steppe in the Crau area (Buisson and Dutoit, 2006; Saatkamp et al., 2017). These different parameters, Mediterranean climate, strong northwesterly winds, high stone cover, and two millennia of extensive grazing, all participate to shape this ecosystem.

We selected four study sites locally named in French "*Coucou, Négreiron, Petit Carton*, and *Le Merle*," along a South-West/North-East gradient following the geomorphological-pedological-climatic gradient to cover all the environmental variability in the area (climate, topography, geology, pedology, and water accessibility) and in grazing pressures (Molinier and Tallon, 1950; Tatin et al., 2013; Table 1; Figure 1). An increase in fine elements (clay, silt) relative to decrease of coarse elements (sand, pebbles) has been shown to drive the presence of the biological crust micro-patterns in the south and their absence in the north of the plain (Rieux et al., 1977). Finally, the four sites are grazed by sheep kept in enclosures or led by a shepherd at an average grazing pressure of 400.8 ± 150 grazed day sheep/ha/year (see below, unpublished data from the Agricultural Chamber of the *Bouches-du-Rhône* department and the *Le Merle* Domain). Two out of the four sites had long-term, established ungrazed areas (exclosures): one of approximately 0.2 ha since 1982 at *Le Merle* and another of approximately 3 ha since 2001 at *Négreiron* (Bourrelly et al., 1983; Pilard and Brun, 2001).

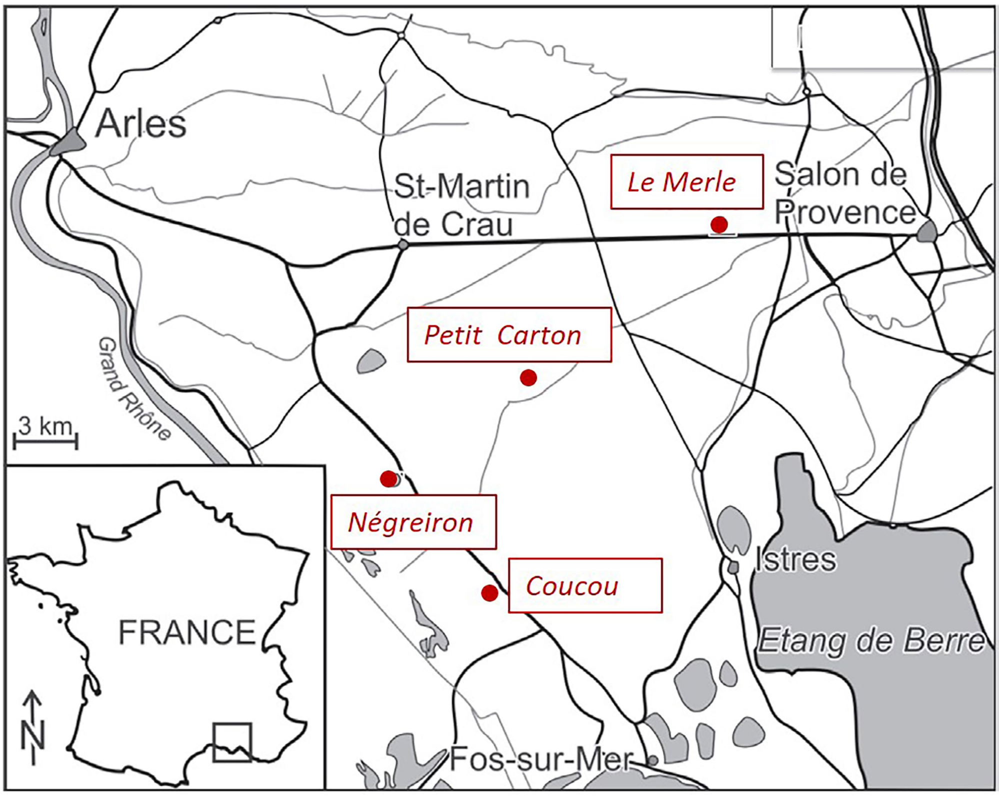


FIGURE 1
Locations of the four study sites in the Crau area, South-East of France.

TABLE 1 Number of plant community relevés carried out for each micro-pattern and site of the Mediterranean dry grassland of the Crau area (South–East France), broken down by treatment (grazed vs. ungrazed in exclosures).

Site	Treatment	Grazing pressure (number of sheep grazing days per ha and year)	Vegetation micro-patterns		
			Biological crusts	Reference communities	Edge communities
Coucou	Grazed	241	10	5	5
Négreiron	Grazed	588	5	5	5
	Ungrazed	0	5	5	0
Petit Carton	Grazed	330	0	5	5
Le Merle	Grazed	444.2	0	5	5
	Ungrazed	0	0	5	0

Grazing pressure is indicated for each site and treatment.

Sampling design and data collection

Within the four sites described above, three micro-patterns in plant communities have been primarily reported: (i) Mediterranean grasslands, locally named "*coussoul*" or *Asphodeletum fistulosi* (*Thero-Brachypodietalia*) following the phytosociological typology by Molinier and Tallon (1950), hereafter named as lightly grazed reference communities, (ii) biological crusts, areas dominated by lichens on shallower soils, locally named "*tonsure*" or *Crassuletum tillaeae* (Tuberarietalia guttati) following the phytosociological typology by Molinier and Tallon (1950), corresponding to present or historically high grazing pressure areas (Rieux et al., 1977), and (iii) edge communities dominated by the tussock perennial grass [*Brachypodium retusum* (Pers.) P.Beauv.] of this grassland, locally named "*grossier*" and associated to the absence of grazing or very low grazing pressure areas, often localized at the edge of grazing plots (Dureau and Bonnefon, 1998). A total of 70 relevés, i.e., a list of each vascular plant species in a community for a defined spatial area, were carried out in the three Mediterranean vegetation micro-patterns: 20 in biological crusts (5 in exclosures), 30 in reference communities (10 in exclosures) and 20 in edge communities (Table 1). This difference in the number of relevés was due to the absence of long-term exclosures at two sites (i.e., no exclosure in sites *Coucou* and *Petit Carton*) or the absence of biological crusts in the north of the plain (i.e., in sites *Le Merle* and *Petit Carton*). Each relevé delimitation was defined taking into account the floristic, structural, and ecological homogeneity necessary to set up a phytosociological survey and on previous phytosociological studies already realized in the Crau area (Molinier and Tallon, 1950; Rieux et al., 1977). A minimum distance of 10 m between relevés located in a same site was observed to avoid any influence of each type of plant community on one another, and relevés were done at least 10 m away from fences (Buisson and Dutoit, 2006; Saatkamp et al., 2017). The floristic data were gathered from April to May 2018, which is the period of the year during which most plants can be identified to the species level in South-East France. All vascular plants were recorded in each relevé, and abundance data were estimated for each species according to the six abundance classes defined by Braun-Blanquet (+, 1, 2, 3, 4, 5; Braun-Blanquet, 1932). The sampled areas of the 70 relevés ranged from 4 to 40 m^2 (average area of grazed biological crusts $8.5 \pm 7.9\,m^2$ vs. ungrazed biological crusts $7.2 \pm 2.2\,m^2$; of grazed reference communities $14.4 \pm 9.2\,m^2$ vs. ungrazed reference communities $6.6 \pm 2.8\,m^2$; of edge communities $13.4 \pm 7.8\,m^2$, sampled area of each relevé are detailed in Supplementary Table A1). The variability of the sampled areas arose from differences in the area occupied by the three different micro-patterns and to their species richness, as we stopped samples when 95% of the species of the plant community was incorporated into the relevé, ratio also based on previous phytosociological surveys realized in the Crau area (Molinier and Tallon, 1950; Rieux et al., 1977). To avoid potential bias, the area was included in all subsequent analyses.

For each relevé, we defined five independent indices that captured the main local environmental parameters: (i) an index of vegetation volume, based on visual estimates of vegetation cover, bare ground, and maximum height of the herbaceous stratum; (ii) a soil fertility index, based on 12 parameters related to soil chemistry (cation exchange capacity, pH water, and pH KCI, available phosphorus, potassium oxide, magnesium oxide, calcium oxide, sodium oxide, total nitrogen, organic carbon, and carbon-to-nitrogen ratio); (iii) an amount of fine soil particles, measured as the amount of clay and fine silt; (iv) an amount of coarse soil particles, i.e., fine sand; and (v) an amount of pebbles (>5 cm), based on visual cover estimates. These indices capture known gradients at the scale of the Crau area (Details in Supplementary Material Appendix I; Supplementary Figure A1) that broadly influence vegetation characteristics. All visual estimations have been collected by the same observer to reduce subjective variability. Grazing pressure was retrieved from inquiries of pastoralists realized by the Agricultural Chamber of the *Bouches-du-Rhône* department and the *Merle* Domain for the four study sites concerning the 55 grazed relevés out of the 70 relevés (15 were located in exclosures) in 2018. We calculated the number of sheep grazing days per ha and per year as Ns * Nd/ha for the grazed season of the year 2018 (autumn 2017 and spring 2018, before the transhumance to the mountain pastures), where Ns the number of sheep in a herd, Nd the number of days of sheep presence on the grazed area, and ha the area of the site (Table 1).

It is important to note that we used two grazing variables. We used first a quantitative variable, described above and named hereafter "grazing pressure." This variable was mostly used in statistical analyses. Second, we compiled a qualitative variable, grazed vs. ungrazed in exclosures, hereafter named "treatment" and used in some graphical representations where appropriate.

Statistical analyses

We calculated the species richness and Pielou's measure of species evenness, hereafter named evenness, which is weakly sensitive to rare taxa, not sensitive to median or dominant taxa, and independent of species richness for sites with more than 20 species (Beisel et al., 2003; Gosselin, 2006). Evenness was calculated as $J = H'/\ln(S)$ where H' is the Shannon diversity index based on the abundance of species in a relevé and S is the total number of species in a relevé.

To explore the possible relationships between the three micro-patterns and the considered response variables, i.e., amount of clay and fine silt, amount of fine sand, soil fertility, pebble cover, vegetation volume, species richness, and evenness, we fitted linear mixed-effects models using the restricted maximum likelihood algorithm as recommended in case of unbalanced design associated to random effect (Pinheiro and Bates, 2000; Bolker et al., 2009). We included three fixed-effect predictors: micro-patterns (qualitative, three levels), grazing pressure (quantitative), and the interaction between both variables, and included the site identity as a random effect on the intercept. We included the logarithm of the sampled area as a control, fixed-effect variable for the models that included species richness, evenness, vegetation volume, and pebble cover as response variables. For the model with pebble cover as response, we did not include grazing pressure and the interaction between grazing pressure and micro-pattern as explanatory variable. Equations are detailed in Supplementary Material Appendix II. We adjusted the p-values obtained from the linear mixed-effects models (Kuznetsova et al., 2017) and from the ANOVA applying the Holm correction (Sinclair et al., 2013). We checked visually using quantile-quantile plots that the residuals were consistent with the assumption of a normal distribution. We calculated the pairwise comparison Wilcoxon tests between micro-pattern group levels according to each response variables with corrections for multiple testing (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001) and between grazed and exclosures micro-patterns.

Multivariate analyses were used to study the relationship between the species composition of plant communities, environmental variables, and grazing pressure. In order to analyze plant community data, Braun-Blanquet scores were transformed into percentage cover on the basis of the average percentage of cover of each class: + = 1%; 1 = 5.5%; 2 = 17.5%; 3 = 37.5%; 4 = 62.5%; 5 = 87.5%. We performed Adonis and pairwise Adonis *post-hoc* tests with Bonferroni adjustments to check whether the plant communities were significantly different between and within groups defined by the micro-pattern, the treatment (grazed vs. exclosure), and the interaction of both variables. Moreover, we implemented a redundancy analysis (RDA) to investigate the variability in plant community composition at the micro-local scale in the Crau area constrained by centered environmental variables and grazing pressure. Note that we considered site and area as conditional variables, to account for the spatial nesting of sampled areas in sites and for the variable area sampled per relevés, respectively. As recommended by Borcard et al. (2011), we Hellinger-transformed the abundance data to reduce the wide disparity in magnitude between species abundances prior to the analysis. We retained all environmental variables (i.e., amount of clay and fine silt, amount of fine sand, soil fertility, and pebble cover) and grazing pressure as explanatory variables according to the Variance Inflation Factor testing collinearity between explanatory variables (all VIF < 5; Borcard et al., 2011). We finally performed a global permutation test to assess the significance of the RDA and a test of the canonical axes significance.

Finally, in order to evaluate and compare direct and indirect effects of grazing versus soil variables on species richness, evenness, and vegetation volume of the three micro-patterns communities, we performed a single multigroup structural equation modeling (SEM), a probabilistic model including multiple predictors and response variables in a single causal network. It provides a means of representing complex hypotheses about causal networks, accounting for factors that are both causes and effects, taking into account interaction effects, and testing for model and data consistency (Grace, 2006; Lefcheck, 2019). We proposed an initial conceptual model containing six regression analyses based on an *a priori* knowledge about the relations among the variables that was both consistent with our data and which made biological sense (Supplementary Figure A2). We considered species richness, vegetation volume, and evenness as endogenous variables, i.e., responses variables, and amount of clay and fine silt, amount of fine sand, soil fertility, pebble cover, and grazing pressure as exogenous variables, i.e., predictors. Note that an endogenous variable can also predict other variables in the model and be characterized simultaneously as endogenous and exogenous variables. This is the case for vegetation volume, species richness, and amount of clay and fine silt which were both endogenous and exogenous variables. A logarithm term of the area was considered as an exogenous variable influencing species richness, vegetation volume, and evenness to account for the variable area sampled per relevé (Supplementary Figure A2). As expected, this term had a direct significant and positive effect on species richness of biological crusts (Standardized estimate = 0.72, value of $p < 0.001$), reference communities (Standardized estimate = 0.3, value of $p < 0.001$), and edge communities (Standardized estimate = 0.6, value of $p < 0.001$), i.e., the larger the sampled area, the higher the species richness. It has not been represented in figures as this variable is a sampling artifact. In each regression analyses of the multigroup SEM, we included an independent random effect to account for the spatial nesting of sampled areas in site. We performed a single multigroup SEM testing relationships between variables for the three micro-patterns of the Mediterranean dry grassland of the Crau area. This single multigroup SEM allows testing for significant differences in the relationships between variables across and between micro-patterns. According to the test of directed separation, no relation was missing in the conceptual model. To evaluate the model and the goodness-of-fit with the data, we used a Fisher's C test (high p-values indicate a good fit, with values well above 0.05 considered acceptable since the null hypothesis here tested is that the data predicted by the model and the observed data are consistent; Lefcheck, 2019).

All analyses and figures were done using R version 3.2.4 (R Development Core Team, 2008), and the packages vegan v2.5.6 (Oksanen et al., 2019), nlme v3.1.142 (Pinheiro et al., 2017), piecewiseSEM v2.1.0 (Lefcheck, 2016), ggplot2 v3.3.1 (Wickham, 2009).

Results

Evidence of differences between the three micro-patterns of dry grasslands

We found differences between the three micro-patterns in terms of species composition. All groups constituted by grazed and ungrazed communities of the three micro-patterns of the Crau area were different from each other in terms of species composition (Pairwise Adonis test, adjusted value of $p < 0.01$; Supplementary Tables A2, A3) except grazed reference communities and grazed edge communities (Pairwise Adonis test, F value = 2.21, adjusted value of $p = 0.41$; Supplementary Tables A2, A3). We found a significant effect of the three micro-patterns (Adonis test, F value = 11.33, value of $p < 0.001$), of the treatment (Adonis test, F-value = 6.82, value of $p < 0.001$) and of the interaction of the both variables micro-patterns and the treatment (Adonis test, F-value = 0.38, value of $p = 0.015$) on the species composition of all groups.

We found differences between the three micro-patterns in terms of soil characteristics, vegetation volume, and species composition. Considering the RDA analysis, permutation tests supported the significance of the RDA (F-value = 4.03, value of $p < 0.001$; Supplementary Table A4): variance in plant community composition was explained to 20.55% by the explanatory variables and to 29.35% by the conditional variables (RDA adjusted $R^2 = 16.4$; Supplementary Table A4). In addition, the first and second canonical axes were significant (Permutation test, RDA 1 F-value = 12.16, value of $p < 0.001$; RDA 2 F-value = 7, value of $p < 0.001$; Supplementary Table A4) and accounted together for 79.17% of the total explained variance. The first RDA axis captured differences in composition of micro-patterns and vegetation volume: it contrasted biological crusts, which had higher level of fine sand and soil fertility, whereas reference communities and edge communities had higher levels of clay, fine silt, and vegetation volume (Figure 2). The second RDA axis separated grazed plant communities from ungrazed plant communities, i.e., exclosures.

We found evidence of differences between the three micro-patterns in terms of species richness, evenness, vegetation volume, and soil characteristics. Linear mixed models showed significantly higher levels of fine sand for biological crusts compared to the other micro-patterns, significantly higher levels of clay and fine silt, of vegetation volume for reference communities and edge communities compared to biological crusts (Table 2; Figure 3; Supplementary Tables A5, A6). Thus, biological crusts were characterized by coarse silt and fine sand, whereas reference communities and edge communities were characterized by a mixture of variables element' size: clay and fine silt and coarse sand. Edge communities were characterized by the lower amount of fine sand in soil than for other micro-patterns (i.e., the higher amount of coarse sand (Figure 3). Finally, linear mixed models did not show significant differences among micro-patterns concerning soil fertility and pebble cover. We found significantly higher levels of clay and fine silt for ungrazed communities compared to grazed communities, higher levels of soil fertility for grazed biological crusts, lower levels of species richness, and evenness for ungrazed reference communities (Supplementary Figure A3).

Micro-patterns exhibit distinct functioning, all of them coexisting at the micro-local scale in dry grasslands

We investigated the direct and indirect effects of grazing pressure and soil variables on species richness, vegetation volume, and evenness of plant communities of the three micro-patterns and the fitting parameters of the adequate multigroup SEM were correct (Fisher's C = 9.75, value of $p = 0.78$, ddl = 14, $n = 70$; Figure 4). This analysis showed that the direct effect of grazing varied across micro-patterns (significant interaction between grazing pressure and micro-patterns against species richness, value of $p < 0.05$), whereas effects of other predictors were similar across micro-patterns (value of $p > 0.05$; Figure 4). Effect sizes revealed three distinct functioning, each one specific of a micro-pattern. Soil variables and grazing had stronger direct and indirect effects on plant communities of biological crusts than on reference communities and edge communities. Direct effects of grazing on species richness differed among each micro-pattern, being negative in biological crusts and positive in reference and edge communities. Finally, soil fertility and pebble cover did not appear to influence plant communities of any micro-patterns according to the multigroup SEM.

Discussion

For the first time, our work disentangles grazing and soil characteristics impacts on micro-pattern patches by showing differences in species composition, vegetation volume, species richness, evenness, and functioning at the micro-local scale among the micro-patterns of a Mediterranean grassland.

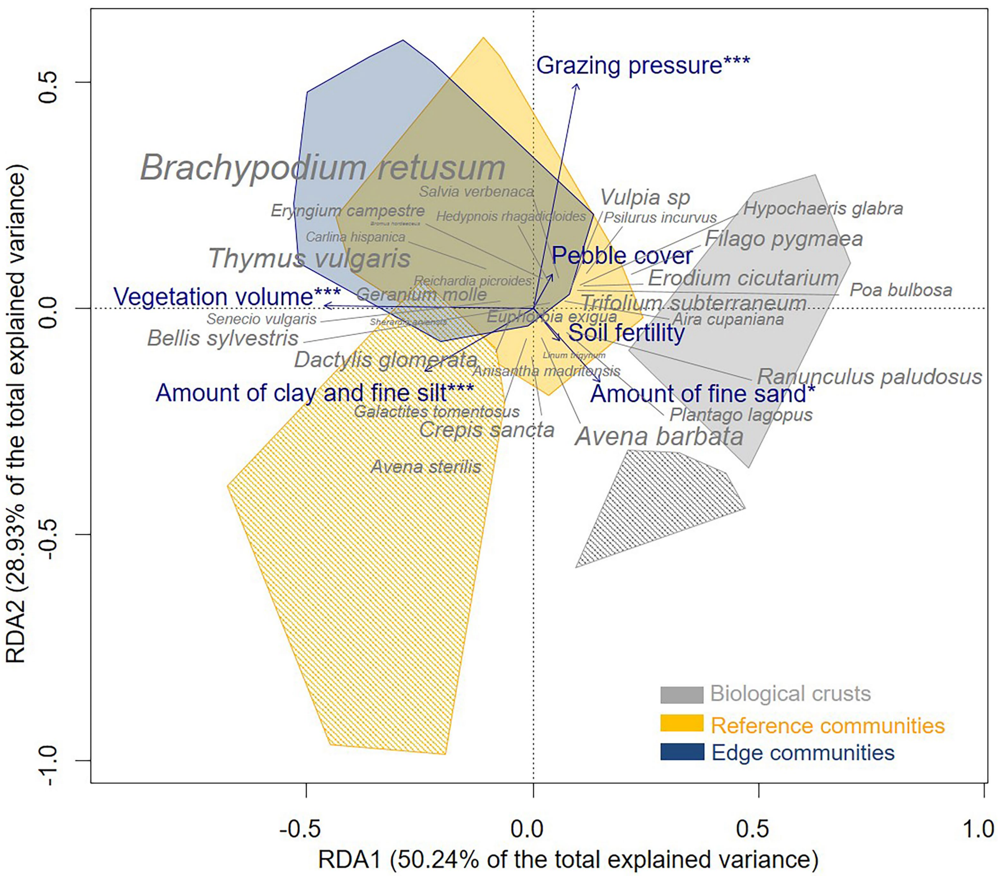


FIGURE 2
Ordination triplot of redundancy analysis (RDA) of the three micro-patterns of the Mediterranean dry grassland of the Crau area (full polygons for grazed relevés, hatched polygons for ungrazed relevés in exclosures) considering grazing and environmental variables (blue, stars precise significance) and two conditional variables (site and sampled area). Only the 30 most abundant species are represented and the size of species' labels is proportional to their frequency.

TABLE 2 **ANOVA Type II (Chi-square tests) and significance level for effects of grazing pressure and micro-patterns against environmental variables, namely amount of clay and fine silt, amount of fine sand, soil fertility, pebble cover, and vegetation volume, species richness, and evenness.**

Effect	df	Amount of clay and fine silt	Amount of fine sand	Soil fertility	Pebble cover	Vegetation volume	Richness	Evenness
Grazing pressure (GP)	1	**18.41***↓**	1.11	0.83	-	**9.18**↓**	**29.31***↑**	**10.09**↑**
Micro-patterns (MP)	2	**22.43*****	15.9**	6.48	1.56	**164.3*****	**8.85***	**29*****
MP x GP	2	0.38	0.06	3.38	-	3.65	**24.6*****	**8.5***

MP × GP, Micro-patterns × Grazing pressure. Factor effect: ↓ decrease; ↑ increase. All parameter estimations are reported and detailed in Supplementary Tables A4A,B. Bold represents significance level.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Origin and maintenance of micro-patterns

This work highlights the contrasted functioning across micro-patterns, as grazing had a variable impact on species richness and soil granulometry. Micro-patterns with higher vegetation cover and volume are those that tend to be less grazed (although this has not been quantified here)—biological crusts are thus likely to be where grazing is the highest, followed by the reference communities and edge communities. Grazing exhibited a negative effect on species richness in biological crusts versus a positive one on richness in reference communities and an indirect and smaller

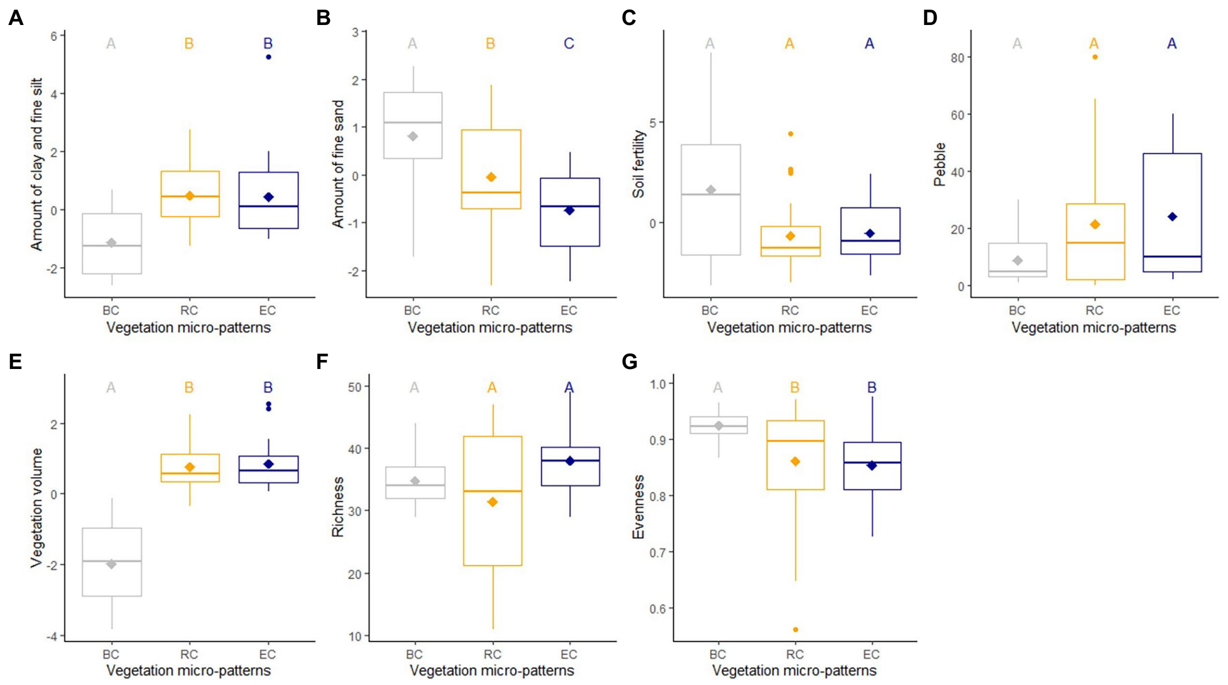


FIGURE 3
Comparison of levels of environmental variables, namely amount of clay and fine silt **(A)**, amount of fine sand **(B)**, soil fertility **(C)**, pebble cover **(D)**; vegetation volume **(E)**, species richness **(F)**, and evenness **(G)** according to the three micro-patterns (BC, biological crusts; RC, reference communities; EC, edge communities). Average (diamond), medians, quartiles, minimum and maximum are colored according to each micro-patterns. Significant differences between averages of each group are indicated with different letters, calculated with pairwise Wilcoxon tests.

positive one on richness of edge communities. This is consistent with the theoretical impact of grazing on species richness—intense grazing decreases diversity (in biological crusts) but lower levels favor diversity, which is what we observe in other micro-patterns (Milchunas et al., 1988; Tatin et al., 2013).

Differences among micro-patterns were particularly stark between biological crusts and the two other micro-patterns, as the former showed very different soil granulometry and vegetation volume. Biological crusts appeared primarily driven by soil variables, and second by grazing. They were more homogeneous in terms of species composition, and had an upper soil containing more fine particles (fine sand, coarse silt) than the other two micro-patterns. Such differences in soil characteristics suggest that biological crust communities may have their origin in present or former grazing practices (Saatkamp et al., 2021). Indeed, soil characteristics are influenced by grazing, suggesting that trampling could be the main driver of local soil differences, which compound with already-existing larger-scale differences, such as the combined geomorphological-pedological-climatic gradient of the Crau area and the spatial heterogeneity of the vegetation (Molinier and Tallon, 1950; Adler et al., 2001; Tatin et al., 2013). Soil trampling and compaction by animals alter the soil structure by compressive deformation: particles are brought closer together by the applied charge, which reduces the total pore space and permanently expels air or water from the soil pores (Patto et al., 1978; Silva et al., 2003). These alterations of the upper part of the soil affect soil physical properties, density, water dynamics, mineral element cycle, and plant growth (Lowery and Schuler, 1991; Schlotzhauer and Price, 1999; De Neve and Hofman, 2000; Drewry et al., 2008). Biological crusts could correspond to past high-impact areas of grazing, which are known to have a long-lasting effect, and to finely textured soils, which are more compressible than others (Larson et al., 1980), especially under stronger grazing pressures. Indeed, the higher amount of fine sand in soil composition could promote biological crusts by compaction, which may impair the installation of the dominant and key species in the Crau area *Brachypodium retusum*. In contrast, the heterogeneity of soil particle sizes in reference and edge communities seems to promote *Brachypodium retusum* establishment, as observed in field. Also, the increased abundance of this perennial graminoid species has been related to lightly grazed edge communities (Porensky et al., 2017). The installation of this key species facilitated by soil grain size characteristics and fire resilience could then result in changes in the microenvironment by improving water infiltration, reducing radiation to the soil, and accumulating seeds, fine soil particles, and nutrients between plant roots (Aguiar and Sala, 1999; Vidaller et al., 2019; Davies et al., 2022). This could also reduce herbivory through a phenomenon of facilitation between species (survival of seedlings protected by the dominant species, Aguiar and Sala, 1999). Taken altogether, these processes could explain the persistence of such contrasted community types at a local scale (<10 m).

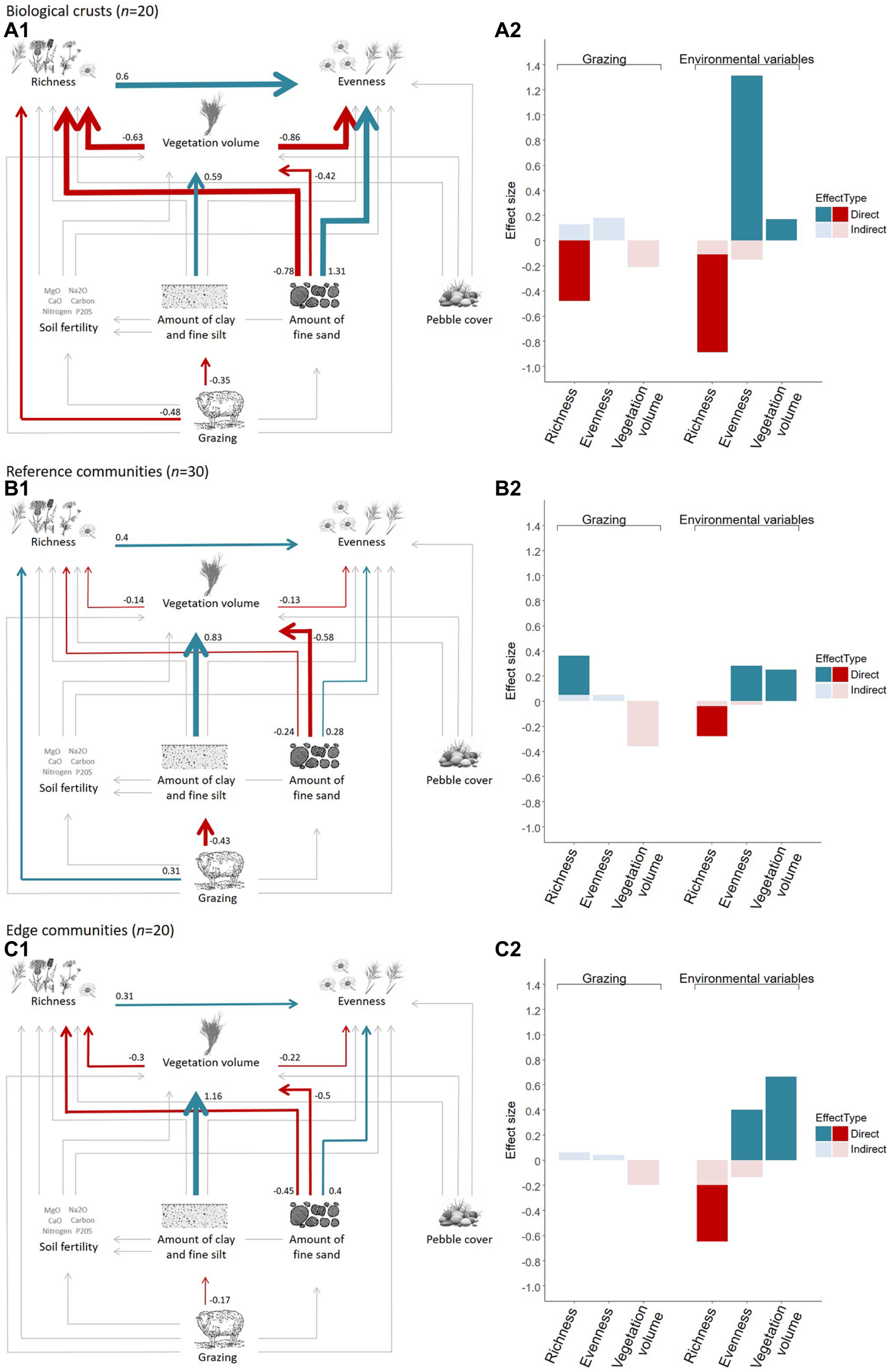


FIGURE 4
Direct and indirect effects of grazing and soil variables on vegetation richness, evenness and volume according to the three micro-patterns of the Mediterranean dry grassland of the Crau area (relations among variables on the left and total direct and indirect effect size on the right resulting from a piecewise multigroup structural equation model, coefficients correspond to standardized estimates). Red (negative effect) and blue (positive effect) arrow widths are proportional to the standardized estimate of each significant effect (numbers along arrows). Gray arrows are non-significant effects.

Reference and edge communities appeared much more similar to each other than to biological crusts, as they had similar species compositions and abundances, species richness, evenness, and vegetation volume, all consequences of variable levels of herbivory (Génin et al., 2021). However, they differ in terms of soil composition (amount of fine sand). Edge communities appeared directly driven by soil variables and in a minor way by grazing, thus contrasting with reference communities which were directly influenced by grazing. Edge communities are often associated with abiotic modifications such as water infiltration (Masson et al., 2015) and not only to low grazing pressure, which does not affect plant community composition unlike high grazing pressure. One notable implication in terms of conservation and restoration is that it is not enough to remove grazing to produce a transition from reference to edge community in the short term because changes must also occur in soil granulometry (i.e., high amount of coarse sands in the soils of edge community). In fact, only long-term grazing removal affects will change the species composition of reference communities into that of edge communities (Saatkamp et al., 2017). Despite being more similar, switching from one reference to edge community (or the other way around) may thus require more than short-term changes in grazing pressure.

No demonstrated effect of pebble cover and soil fertility on micro-patterns

No effect of pebble cover has been found, although it is widely thought to impact positively species richness, vegetation volume, and evenness, by constituting a root shelter allowing the installation of *Brachypodium retusum* (Bourrelly et al., 1983) and other species, improving microenvironmental conditions and reducing herbivory by physically protecting the species from sheep's mouths (Dureau and Bonnefon, 1998; Aguiar and Sala, 1999). This is probably due to the differences in sampled area among micro-pattern (more pebbles in the less grazed areas are also the largest sampled areas), which is not independent from the variable size and density of pebble related to the gradient of the alluvial deposits of the Durance river (Devaux et al., 1983), the largest pebbles at the highest recovery being encountered in the north-west where we did not sample biological crusts (*Le Merle* site; Figure 1), while the fine elements in the superficial part of the soil are more present in the south of the Crau area (*Coucou* site; Figure 1). Likewise, no effect of soil fertility has been found and no difference among each micro-pattern even though the former over-grazed areas could be suspected to be characterized by accumulation of nutrients and animal waste, beneficial to plant growth, long-term fertility, and known to cause a shift in community diversity and composition (Matches, 1992; Liu et al., 2016). These persistent legacies of historical sheep concentration, as Roman ones, on soil properties have persistent effects on today's vegetation in this area (Saatkamp et al., 2021).

Consequences for biological conservation and restoration of mediterranean dry grasslands

This work showed that ecosystem functioning varies over very small spatial scales: the three distinct communities at the micro-local scale are maintained by different direct and indirect effects of grazing and soil characteristics. Thus, there is no one-size-fits-all strategy to influencing the trajectory of the plant communities and one of the main levers of restoration strategies, changing grazing pressure, may have variable effects depending on the community type it is applied to. Grazing has a long-term residual effect on plant communities and soils, so it is not sufficient to remove it in the short term to restore a particular Mediterranean dry grassland community (Buisson et al., 2020). Indeed, grazing-induced changes in soil characteristics and fertility *via* long-term persistent eutrophication (soil phosphorus, calcium, and nitrogen) and trampling have effects on historical time-scales, as there is little opportunity to uncompact soil structure (Greenwood and McKenzie, 2001; Saatkamp et al., 2021). Grazing thus acts as a one-way lever: given the relative similarity between reference and edge community, it is likely that increased grazing pressure in edge communities would make them shift toward reference communities. However, the sole removal of grazing in reference communities is unlikely to drive them into edge-type communities on short time-scales, because of the need to reverse soil characteristics too. A similar and more pronounced effect is also likely in biological crusts: driving them toward other community types would require undoing the high soil compaction, which only occurs on very long time-scales (centuries) without drastic human intervention. Indeed, effect of past sheepfolds and intense grazing are still detectable on vegetation and soils, even after two millennia (Saatkamp et al., 2021).

In this situation, there is little opportunity to reverse such effects for restoration purposes. However, authors have already shown that a species of harvesting ant, *Messor barbarus,* could play an important role in the restoration of plant communities in the Crau area, by displacing a large quantity of plant seeds and changing physical and chemical soil characteristics toward that of the reference communities (Cramer et al., 2008; García-Ruiz and Lana-Renault, 2011; De Almeida et al., 2020). Typical dry grassland species are known to have low capacity to colonize new habitats or persist in the soil seed bank (Buisson et al., 2006; Metzner et al., 2017). Thus, increasing pebble cover and re-introducing *Messor barbarus* may provide an interesting and low-cost tool to reverse compaction and disperse seeds (Bulot et al., 2014; De Almeida et al., 2020).

In this work, we presented the first overview of the multiple effects of grazing and soil characteristics on plant communities of micro-patterns at the micro-local scale. Our results should enable conservation planners to use grazing and/or ecological engineer species as effective tools to influence plant community composition and soil structure. A more complete picture may emerge by measuring additional processes influencing the distribution of micro-patterns such as water infiltration capacity in case of compacted soil, wind-based redistribution of seeds, and fine soil elements that may accumulate in tall vegetation patches, and fire (Burke et al., 1998; Aguiar and Sala, 1999; Vidaller et al., 2019). Evaluating these additional effects would bring an understanding of the governing forces that shape community assembly in the context of Mediterranean dry grasslands conservation and restoration (Török et al., 2018).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author. Full plant and soil data areavailable in the open repository Zenodo: https://doi.org/10.5281/zenodo.7222092.

Author contributions

AC, TD, and GM conceived the research idea. AC, HR, and TD collected data. GM performed statistical analyses. GM, with contributions from TD and AG wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grants from the French Ministry of Ecological and Solidarity Transition, i.e., *Ministère de la Transition Écologique et Solidaire*, within the framework of the national research project (CARHAB – project no. 2101536326, *Approche multi-scalaire de l'organisation des parcours substeppiques des Thero-Brachypodietea dans le sud-est de la France: apport à la compréhension des liens entre géocurtaséries et impacts anthropiques actuels et/ou passés*. Received by TD). AG has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 896159.

Acknowledgments

We thank Frédéric Bioret for his help initiating this project and Tania de Almeida, Elise Buisson, Julie Chenot, Aure Durbecq, Cannelle Moinardeau, and Daniel Pavon for their help with collecting floristic and soil data; Gaelle Besche and Fanny Sauguet for their help with obtaining grazing data; Christel Vidaller, Armin Bischoff, Olivier Blight, François Duchenne, and Mathieu Santonja for their constructive comments and advice during the data analyses; and the Federal State Nature Conservation Authority (CEN-PACA), the Crau nature reserve, and the *Le Merle* Domain and training center for shepherds for their permission to set up the experiments. We also thank two reviewers and one editor for their comments on a previous version of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.879060/full#supplementary-material

References

Adler, P., Raff, D., and Lauenroth, W. (2001). The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* 128, 465–479. doi: 10.1007/s004420100737

Aguiar, M. R., and Sala, O. E. (1999). Patch structure, dynamics and implications for the functioning of arid ecosystems. *Trends Ecol. Evol.* 14, 273–277. doi: 10.1016/S0169-5347(99)01612-2

Badan, O., Brun, J.-P., and Congès, G. (1995). Les bergeries romaines de La Crau d'Arles: Les origines de la transhumance en Provence. *Gallia* 52, 263–310. doi: 10.3406/galia.1995.3152

Bakker, J. P., de Leeuw, J., and van Wieren, S. E. (1983). Micro-patterns in grassland vegetation created and sustained by sheep-grazing. *Vegetatio* 55, 153–161. doi: 10.1007/BF00045017

Beisel, J.-N., Usseglio-Polatera, P., Bachmann, V., and Moreteau, J.-C. (2003). A comparative analysis of evenness index sensitivity. *Internat. Rev. Hydrobiol.* 88, 3–15. doi: 10.1002/iroh.200390004

Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc Series B* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Benjamini, Y., and Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* 29, 1165–1188. doi: 10.1214/aos/1013699998

Bissardon, M., Guibal, L., and Rameau, J.-C. (1997). *CORINE biotopes. Types d'habitats français* Paris, France: ENGREF, MNHN.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008

Bond, W., and Keeley, J. (2005). Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends Ecol. Evol.* 20, 387–394. doi: 10.1016/j.tree.2005.04.025

Borcard, D., Gillet, F., and Legendre, P. (2011). *Numerical ecology with R*. New York Dordrecht London Heidelberg: Springer.

Bourrelly, M., Borel, L., Devaux, J., Louis-Palluel, J., and Archiloque, A. (1983). Dynamique annuelle et production primaire nette de l'écosystème steppique de Crau (Bouches du Rhône). *Biologie et écologie méditerranéenne* 10, 55–82.

Braun-Blanquet, J. (1932). *Plant Sociology. The Study of Plant Communities*. United States of America McGraw-Hill Book Co., Inc., New York and London, pp.xviii + 439.

Buisson, E., De Almeida, T., Durbecq, A., Arruda, A. J., Vidaller, C., Alignan, J., et al. (2020). Key issues in northwestern Mediterranean dry grassland restoration. *Restor. Ecol.* 29:rec.13258. doi: 10.1111/rec.13258

Buisson, E., and Dutoit, T. (2006). Creation of the natural reserve of La Crau: implications for the creation and management of protected areas. *J. Environ. Manage.* 80, 318–326. doi: 10.1016/j.jenvman.2005.09.013

Buisson, E., Dutoit, T., Torre, F., Römermann, C., and Poschlod, P. (2006). The implications of seed rain and seed bank patterns for plant succession at the edges of abandoned fields in Mediterranean landscapes. *Agric. Ecosyst. Environ.* 115, 6–14. doi: 10.1016/j.agee.2005.12.003

Bulot, A., Provost, E., and Dutoit, T. (2014). A comparison of different soil transfer strategies for restoring a Mediterranean steppe after a pipeline leak (La Crau plain, south-eastern France). *Ecol. Eng.* 71, 690–702. doi: 10.1016/j.ecoleng.2014.07.060

Burke, I. C., Lauenroth, W. K., Vinton, M. A., Hook, P. B., Kelly, R. H., Epstein, H. E., et al. (1998). "Plant-soil interactions in temperate grasslands," in *Plant-induced soil changes: Processes and feedbacks*, ed. BreemenN. Van (Dordrecht: Springer Netherlands), 121–143.

Chenot, J., Gaget, E., Moinardeau, C., Jaunatre, R., Buisson, E., and Dutoit, T. (2017). Substrate composition and depth affect soil moisture behavior and plant-soil relationship on Mediterranean extensive green roofs. *Water* 9:817. doi: 10.3390/w9110817

Coiffait-Gombault, C., Buisson, E., and Dutoit, T. (2012). Are old Mediterranean grasslands resilient to human disturbances? *Acta Oecol.* 43, 86–94. doi: 10.1016/j.actao.2012.04.011

Cramer, V., Hobbs, R., and Standish, R. (2008). What's new about old fields? Land abandonment and ecosystem assembly. *Trends Ecol. Evol.* 23, 104–112. doi: 10.1016/j.tree.2007.10.005

Dantas, V. L., Hirota, M., Oliveira, R. S., and Pausas, J. G. (2016). Disturbance maintains alternative biome states. *Ecol. Lett.* 19, 12–19. doi: 10.1111/ele.12537

Davies, K. W., Copeland, S. M., and Bates, J. D. (2022). Grazing effects on shrub-induced resource islands and herbaceous vegetation heterogeneity in sagebrush-steppe communities. *Glob. Ecol. Conserv.* 35:e02106. doi: 10.1016/j.gecco.2022.e02106

De Almeida, T., Blight, O., Mesléard, F., Bulot, A., Provost, E., and Dutoit, T. (2020). Harvester ants as ecological engineers for Mediterranean grassland restoration: impacts on soil and vegetation. *Biol. Conserv.* 245:108547. doi: 10.1016/j.biocon.2020.108547

De Neve, S., and Hofman, G. (2000). Influence of soil compaction on carbon and nitrogen mineralization of soil organic matter and crop residues. *Biol. Fertil. Soils* 30, 544–549. doi: 10.1007/s003740050034

Devaux, J., Archiloque, A., Borel, L., Bourrelly, M., and Louis-Alluel, J. (1983). Notice de la carte phytoécologique de la Crau (Bouches-du-Rhône). *Biologie Ecologie Méditerranéenne*, 10, 5–54.

Drewry, J. J., Cameron, K. C., and Buchan, G. D. (2008). Pasture yield and soil physical property responses to soil compaction from treading and grazing—a review. *Soil Res.* 46:237. doi: 10.1071/SR07125

Dureau, R., and Bonnefon, O. (1998). "Etude des pratiques de gestion pastorale des coussouls" in *Patrimoine naturel et pratiques pastorales en Crau*, CEN-PACA, CA 13, Saint-Martin de Crau, France. 61–89.

Eurostat (2017). Land cover and land use, landscape (LUCAS). Available at: http://ec.europa.eu/eurostat/web/lucas/data/database.

García-Ruiz, J. M., and Lana-Renault, N. (2011). Hydrological and erosive consequences of farmland abandonment in Europe, with special reference to the Mediterranean region – a review. *Agric. Ecosyst. Environ.* 140, 317–338. doi: 10.1016/j.agee.2011.01.003

Ge, Z., and Liu, Q. (2022). Foraging behaviours lead to spatiotemporal self-similar dynamics in grazing ecosystems. *Ecol. Lett.* 25, 378–390. doi: 10.1111/ele.13928

Génin, A., Dutoit, T., Danet, A., le Priol, A., and Kéfi, S. (2021). Grazing and the vanishing complexity of plant association networks in grasslands. *Oikos* 130, 541–552. doi: 10.1111/oik.07850

Gibson, D. J. (2009). *Grasses and grassland ecology* Oxford, UK: Oxford University Press.

Gosselin, F. (2006). An assessment of the dependence of evenness indices on species richness. *J. Theor. Biol.* 242, 591–597. doi: 10.1016/j.jtbi.2006.04.017

Grace, J. B. (2006). *Structural equation modeling and natural systems*. Cambridge University Press. UK.

Greenwood, K. L., and McKenzie, B. M. (2001). Grazing effects on soil physical properties and the consequences for pastures: a review. *Aust. J. Exp. Agric.* 41, 1231–1250. doi: 10.1071/ea00102

Helm, J., Dutoit, T., Saatkamp, A., Bucher, S. F., Leiterer, M., and Römermann, C. (2019). Recovery of Mediterranean steppe vegetation after cultivation: legacy effects on plant composition, soil properties and functional traits. *Appl. Veg. Sci.* 22, 71–84. doi: 10.1111/avsc.12415

Henry, F., Talon, B., and Dutoit, T. (2010). The age and history of the French Mediterranean steppe revisited by soil wood charcoal analysis. *Holocene* 20, 25–34. doi: 10.1177/0959683609348841

Jacobs, B. F., Kingston, J. D., and Jacobs, L. L. (1999). The origin of grass-dominated ecosystems. *Ann. Mo. Bot. Gard.* 86:590. doi: 10.2307/2666186

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Softw.* 82, 1–26. doi: 10.18637/jss.v082.i13

Larson, W. E., Gupta, S. C., and Useche, R. A. (1980). Compression of agricultural soils from eight soil orders. *Soil Sci. Soc. Am. J.* 44, 450–457. doi: 10.2136/sssaj1980.03615995004400030002x

Lefcheck, J. (2016). Piecewise SEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods Ecol. Evol.* 7, 573–579. doi: 10.1111/2041-210X.12512

Lefcheck, J. (2019). Structural equation modeling in R for ecology and evolution. Available at: https://jslefche.github.io/sem_book/index.html

Liu, C., Song, X., Wang, L., Wang, D., Zhou, X., Liu, J., et al. (2016). Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore assemblage and pre-grazing plant diversity. *J. Appl. Ecol.* 53, 242–250. doi: 10.1111/1365-2664.12537

Louvel, J., Gaudillat, V., and Poncet, L. (2013). *EUNIS, European Nature Information System, Système d'information européen sur la nature. Classification des habitats. Traduction française. Habitats terrestres et d'eau douce* Paris, France: MNHN-DIREV-SPN, MEDDE.

Lowery, B., and Schuler, R. T. (1991). Temporal effects of subsoil compaction on soil strength and plant growth. *Soil Sci. Soc. Am. J.* 55, 216–223. doi: 10.2136/sssaj1991.03615995005500010037x

Masson, S., Gauvain, M., Mesléard, F., and Dutoit, T. (2015). Impacts of water stress removal and disturbance regimes on Mediterranean dry grasslands diversity and succession. *Plant Ecol.* 216, 1351–1369. doi: 10.1007/s11258-015-0513-5

Matches, A. G. (1992). Plant response to grazing: a review. *J. Prod. Agric.* 5, 1–7. doi: 10.2134/jpa1992.0001

McNaughton, S. J. (1983). Serengeti grassland ecology: the role of composite environmental factors and contingency in community organization. *Ecol. Monogr.* 53, 291–320. doi: 10.2307/1942533

Metzner, K., Gachet, S., Rocarpin, P., and Saatkamp, A. (2017). Seed bank, seed size and dispersal in moisture gradients of temporary pools in southern France. *Basic Appl. Ecol.* 21, 13–22. doi: 10.1016/j.baae.2017.06.003

Milchunas, D. G., and Lauenroth, W. K. (1993). Quantitative effects of grazing on vegetation and soils over a global range of environments: ecological archives M063-001. *Ecol. Monogr.* 63, 327–366. doi: 10.2307/2937150

Milchunas, D. G., Sala, O. E., and Lauenroth, W. K. (1988). A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132, 87–106. doi: 10.1086/284839

Molinier, R., and Tallon, G. (1950). La végétation de la Crau (Basse-Provence). *Revue Générale de Botanique*, 1, 1–111.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., et al. (2019). Vegan: community ecology package. Available at: https://CRAN.R-project.org/package=vegan

Patto, P. M., Clement, C. R., and Forbes, T. J. (1978). *Grassland Poaching in England and Wales*. Grassland Research Institute. Maidenhead.

Pfeiffer, M., Langan, L., Linstädter, A., Martens, C., Gaillard, C., Ruppert, J. C., et al. (2019). Grazing and aridity reduce perennial grass abundance in semi-arid rangelands – insights from a trait-based dynamic vegetation model. *Ecol. Model.* 395, 11–22. doi: 10.1016/j.ecolmodel.2018.12.013

Pilard, P., and Brun, L. (2001). *Guide de gestion des milieux en faveur du Faucon crécerellette de la plaine de Crau* Saint-Martin de Crau , Unpublished report.

Pinheiro, J. C., and Bates, D. M. (2000). *Mixed-effects models in S and S-PLUS*. New York: Springer.

Pinheiro, J. C., Bates, D. M., Deb Roy, S., and Sarkar, D.R Development Core Team (2017). nlme: Linear and Nonlinear Mixed Effects Models. Available at: https://CRAN.R-project.org/package=nlme.

Porensky, L. M., Derner, J. D., Augustine, D. J., and Milchunas, D. G. (2017). Plant community composition after 75 Yr of sustained grazing intensity treatments in shortgrass steppe. *Rangel. Ecol. Manage.* 70, 456–464. doi: 10.1016/j.rama.2016.12.001

Poschlod, P., and Baumann, A. (2010). The historical dynamics of calcareous grasslands in the central and southern Franconian Jurassic mountains: a comparative pedoanthracological and pollen analytical study. *Holocene* 20, 13–23. doi: 10.1177/0959683609348843

R Development Core Team. (2008). R: A language and environment for statistical computing. Available at: http://www.R-project.org

Rieux, R., Ritschel, G., and Roux, C. (1977). Etude écologique et phytosociologique de *Crassuletum tillaeae* Molinier et Tallon 1949. *Revue de biologie et d'écologie méditerranéenne* 6, 117–143.

Robin, V., Nelle, O., Talon, B., Poschlod, P., Schwartz, D., Bal, M.-C., et al. (2018). A comparative review of soil charcoal data: spatiotemporal patterns of origin and long-term dynamics of Western European nutrient-poor grasslands. *Holocene* 28, 1313–1324. doi: 10.1177/0959683618771496

Römermann, C., Dutoit, T., Poschlod, P., and Buisson, E. (2005). Influence of former cultivation on the unique Mediterranean steppe of France and consequences for conservation management. *Biol. Conserv.* 121, 21–33. doi: 10.1016/j.biocon.2004.04.006

Saatkamp, A., Henry, F., and Dutoit, T. (2017). Vegetation and soil seed bank in a 23-year grazing exclusion chronosequence in a Mediterranean dry grassland. *Plant Biosyst.* 152, 1020–1030. doi: 10.1080/11263504.2017.1407375

Saatkamp, A., Henry, F., and Dutoit, T. (2021). Romans shape Today's vegetation and soils: two millennia of land-use legacy dynamics in Mediterranean grasslands. *Ecosystems* 24, 1268–1280. doi: 10.1007/s10021-020-00581-w

San Miguel, A. (2008). *Management of natura 2000 habitats. 6220* pseudo-steppe with grasses and annuals of the Thero-Brachypodietea ATECMA,* Spain: European Commission.

Schlotzhauer, S. M., and Price, J. S. (1999). Soil water flow dynamics in a managed cutover peat field, Quebec: field and laboratory investigations. *Water Resour. Res.* 35, 3675–3683. doi: 10.1029/1999WR900126

Silva, A., Imhoff, S., and Corsi, M. (2003). Evaluation of soil compaction in an irrigated short-duration grazing system. *Soil Tillage Res.* 70, 83–90. doi: 10.1016/S0167-1987(02)00122-8

Sinclair, J. K., Taylor, P. J., and Hobbs, S. J. (2013). Alpha level adjustments for multiple dependent variable analyses and their applicability – a review. *Int. J. Sports Sci. Eng.* 7, 17–20.

Suttie, J. M., Reynolds, S. G., and Batello, C. (2005). *Grasslands of the world* , FAO, Rome, Italy. Food and Agriculture Org.

Tatin, L., Wolff, A., Boutin, J., Colliot, E., and Dutoit, T. (2013). *Écologie et conservation d'une steppe méditerranéenne: La plaine de la Crau , Versailles,* France: Quae éditions Edn.

Török, P., Helm, A., Kiehl, K., Buisson, E., and Valkó, O. (2018). Beyond the species pool: modification of species dispersal, establishment, and assembly by habitat restoration: restoration modify dispersal and establishment. *Restor. Ecol.* 26, S65–S72. doi: 10.1111/rec.12825

Veldman, J. W., Buisson, E., Durigan, G., Fernandes, G. W., Le Stradic, S., Mahy, G., et al. (2015). Toward an old-growth concept for grasslands, savannas, and woodlands. *Front. Ecol. Environ.* 13, 154–162. doi: 10.1890/140270

Vidaller, C., Dutoit, T., Ramone, H., and Bischoff, A. (2019). Fire increases the reproduction of the dominant grass *Brachypodium retusum* and Mediterranean steppe diversity in a combined burning and grazing experiment. *Appl. Veg. Sci.* 22, 127–137. doi: 10.1111/avsc.12418

White, R. P., Murray, S., and Rohweder, M. (2000). *Pilot Analysis of Global Ecosystems: Grassland Ecosystems*. Washington, DC: World Resources Institute.

Wickham, H. (2009). *Ggplot2: Elegant Graphics for Data Analysis.* Dordrecht, NL: Springer.

Wolff, A., Tatin, L., and Sauguet, F. (2015). La réserve naturelle des Coussouls de Crau. *Plan de gestion*, Saint-Martin de Crau, France: Internal document, CEN-PACA. 2015–2024.

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 16 January 2023
DOI 10.3389/fevo.2022.1061484

Long-term success of floodplain meadow restoration on species-poor grassland

Leonhard Sommer[1]*, Yves P. Klinger[1], Tobias W. Donath[2], Till Kleinebecker[1] and Sarah Harvolk-Schöning[1]

[1]Division of Landscape Ecology and Landscape Planning, Department of Landscape Ecology and Resources Management, Justus Liebig University Giessen, Giessen, Germany, [2]Department of Landscape Ecology, Institute for Natural Resource Conservation, Kiel University, Kiel, Germany

EDITED BY
Péter Török,
University of Debrecen,
Hungary

REVIEWED BY
László Erdős,
Hungarian Academy of Science,
Hungary
Stephen Venn,
University of Łódź,
Poland

*CORRESPONDENCE
Leonhard Sommer
✉ leonhard.sommer@umwelt.uni-giessen.de

SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 04 October 2022
ACCEPTED 28 December 2022
PUBLISHED 16 January 2023

CITATION
Sommer L, Klinger YP, Donath TW, Kleinebecker T and Harvolk-Schöning S (2023) Long-term success of floodplain meadow restoration on species-poor grassland. *Front. Ecol. Evol.* 10:1061484.
doi: 10.3389/fevo.2022.1061484

Restoration of floodplain meadows remains a challenge, as many degraded sites suffer from seed limitation. The transfer of seed-containing plant material from species-rich donor sites is a widely used method to restore semi-natural grasslands. However, most studies on the success of such restoration projects comprise limited time frames. As factors determining restoration success may only become evident after many years, long-term observations are crucial. We re-investigated 20 restored grassland sites in the floodplain of the Northern Upper Rhine 13–16 years after plant material transfer with different soil preparation treatments. To this end, we carried out vegetation surveys on 254 permanent plots and studied the potential influence of soil preparation, soil nutrients, and hydrology on plant species composition, diversity, and transfer of target species. Since sustainable agricultural use is important to ensure the long-term stability of restored semi-natural grasslands, we further investigated biomass productivity and feeding value. While most target species increased in frequency or remained stable over time, we found no positive long-term effect of soil preparation on vegetation development and target species establishment. Instead, increased biomass yield and flooding frequency led to reduced restoration success, while higher soil C/N ratios had a positive effect. Overall, restoration measures did not affect the agricultural value of the restored grasslands, which had higher dry matter biomass yields compared with the donor sites. Our results indicate that the positive effect of soil preparation on the number and cover of target species, which is regularly reported in short-term studies, diminishes over time, and other factors such as site conditions become increasingly important. Furthermore, additional plant material transfer or manual seeding may be necessary to support target species establishment. Concerning agricultural usability, the integration of restored floodplain meadows in farming systems is possible and can ensure long-term management and thus stability of these ecosystems. Our study shows that long-term monitoring of restoration projects is necessary, as factors determining restoration success may only become evident in the long-term.

KEYWORDS

conservation, farming, feeding value, green hay transfer, plant material transfer, seed limitation, soil disturbance

Introduction

The worldwide degradation of ecosystems is one of the most urgent problems of our time (Díaz et al., 2019). Ecological restoration is a major tool to counteract ecosystem degradation, helping the health, integrity, and sustainability of ecosystems to recover (Society for Ecological Restoration, 2004; IPBES, 2019). The current UN "Decade on ecosystem restoration" (UN General Assembly, 2019) underpins the increasing importance of this field. Growing focus is given to the conservation and restoration of grasslands, as they cover a large proportion of land surface and provide high capacity to support biodiversity, multiple ecosystem services and are integral to human well-being (Bardgett et al., 2021). In Central Europe, semi-natural grasslands are of particular importance. They are the result of centuries of human activity, and low-intensive management by mowing or grazing is required to restore and maintain these semi-natural ecosystems and the services they provide (Bakker, 1989; Hejcman et al., 2013).

Floodplain meadows are outstandingly diverse grassland ecosystems with many rare and endangered plant species (Rodwell, 1992; Wesche et al., 2012). Historically, due to their high productivity, they served as an important source for forage provision for livestock (Rothero et al., 2016). Over the last decades, massive structural changes in agriculture resulted in severe consequences for floodplain meadows (Jefferson and Pinches, 2009). Conversion to arable fields, fertilisation, higher cutting frequencies, and alterations of the hydrological conditions have led to a drastic decline both in the amount of floodplain meadows and their ecological quality (Böger, 1991; Joyce and Wade, 1998; Bissels et al., 2004). The restoration of the plant diversity of floodplain meadows is therefore urgent, but is a challenging and long-lasting process (Engst et al., 2016).

The mere return to low-intensive management on degraded floodplain meadows often fails, as typical plant species hardly re-establish spontaneously. This is due to the transient soil seed bank of many typical floodplain meadow species (Bekker et al., 2000; Hölzel and Otte, 2004a) and lacking connectivity to the few species-rich remnant populations (Donath et al., 2003; Bissels et al., 2004). Therefore, active diaspore introduction is required to re-establish the typical vegetation within a reasonable timespan (Bissels et al., 2004; Vécrin et al., 2007; Jõgar and Moora, 2008; Ludewig et al., 2021). Research projects have shown the suitability of active species introduction for grassland restoration (Kiehl et al., 2010). Out of the available methods, the transfer of freshly cut plant material is considered particularly advantageous with respect to genetic diversity and autochthonism, and additionally enables the transfer of organisms other than plants, such as invertebrates (Harnisch et al., 2014; Stöckli et al., 2021).

Generally, the restoration of species-rich grassland using freshly cut plant material is more challenging on species-poor grassland sites compared to arable fields or raw soils (Kiehl et al., 2010; Hansen et al., 2022; Valkó et al., 2022). Soil preparation is commonly regarded as an important prerequisite for successful target species introduction. While it reduces competition by the existing grassland vegetation and creates niches for germination and successful establishment of seedlings with low competitive power (Schmiede et al., 2012), there is increasing evidence that its positive effects can diminish over longer time periods (Harvolk-Schöning et al., 2020; Freitag et al., 2021). This affirms the importance of long-term monitoring to evaluate the success of restoration measures (Resch et al., 2019), as well as considering a range of driving factors (Hölzel, 2019).

However, in addition to restoration, semi-natural grasslands require adapted management to create adequate disturbance regimes and to overcome seed limitation (Klinger et al., 2021). Typically, management of floodplain meadows consists of mowing, which was traditionally complemented by grazing in some areas (Kapfer, 2010). To ensure an adequate management, farmers often receive subsidies as part of agri-environment schemes (Donath et al., 2021; European Environment Agency, 2022). However, the acceptance for low-intensity management practices might be increased if the biomass produced on these sites could be used profitably. Thus it is desirable to keep floodplain meadows integrated in the regional farming systems (Tallowin and Jefferson, 1999; Donath et al., 2015). This was commonly the case until the middle of the last century, but with more possibilities to increase productivity, e.g., by fertiliser input, the interest of farmers to continue this practice decreased (Hejcman et al., 2013). If it could be shown that species diversity and composition had neutral or positive effects on fodder quantity and/or quality, this might increase the motivation of farmers to re-establish the management of sites with high nature conservation value (Donath et al., 2015). Donath et al. (2015) found that in comparison to sites with low nature conservation value, the fodder quality was comparable or even higher in sites with high nature conservation value, and that the harvested material could be integrated in farming systems. If this were the case also for restored semi-natural grasslands, a sustainable management of these sites and thus long-term restoration success could be ensured easier.

Soil conditions and productivity of floodplain meadows are linked to their agricultural value, which may consequently result in a conflict of goals for any restoration efforts (Donath et al., 2015). Increased nutrient levels can hamper the establishment of target species (Gough and Marrs, 1990; Pywell et al., 2006; Waldén and Lindborg, 2016), but relevant nutrients and respective thresholds vary between study systems. In addition, nutrient stoichiometry can modify restoration outcomes, as, e.g., limitation by nitrogen (N) has been shown to compensate for negative impacts of high P and K availability, restricting productivity and species competition (Pywell et al., 2002; Donath et al., 2007). Additionally, hydrological conditions such as flood and drought frequencies can strongly affect species composition in floodplains (Hölzel, 1999; Mathar et al., 2015), but their impact on restoration success has barely been studied so far.

In a large-scale floodplain meadow restoration experiment with plant material transfer at the Northern Upper Rhine in Germany, the effect of soil preparation and soil properties on species establishment on species-poor grassland had been investigated over the first 3 years (Schmiede et al., 2012). Here, we re-investigated the sites 13–16 years after the restoration to answer the following questions:

a. How have the target species developed on the restoration sites, and is the effect of soil preparation on species richness and composition still detectable after 13–16 years?
b. Do the restoration sites differ from the donor sites and the unrestored grassland in the close surrounding with respect to their ecological properties?
c. What is the agricultural value of the restoration sites, compared to unrestored reference grassland in the surrounding and the donor sites?
d. Which effect do soil properties, productivity, nutrient stoichiometry, and hydrological characteristics of the restoration sites have on the long-term restoration success?

Materials and methods

Study site

The study area is located approximately 30 km southwest of Frankfurt in Hesse (Germany), in the floodplain of the Northern Upper Rhine. The mean annual temperature of 11.1°C marks the region as one of the warmest in Germany, and the mean annual precipitation is relatively low with 550 mm (HLNUG, 2022; stations Frankfurt (Main) Airport for temperature and Groß-Gerau-Wallerstädten for precipitation, 1992–2021). The fluctuating water level of the river Rhine results in both floods and droughts, with groundwater levels of up to 5 m below the surface (HLNUG, 2021). Soils are characterised by high clay contents often exceeding 50% (Burmeier et al., 2010), which adds to the alternating soil water conditions. The specific site conditions and low-intensive haymaking supported the development of species-rich floodplain meadows of the alliances *Molinion* and *Cnidion* (habitat types 6410 and 6440 according to the EU Fauna-Flora-Habitat directive), containing a high number of rare and endangered (alluvial) grassland species (Hölzel, 1999; Donath et al., 2003). However, intensification and conversion into arable fields caused massive habitat losses in the course of the 20th century, leaving only small isolated remnants of species-rich floodplain meadows (Böger, 1991; Hölzel and Otte, 2003).

Restoration sites and measures

After a series of major floods in 1983, 150 ha of arable fields were converted to non-intensively managed, unfertilized grassland in order to re-establish species-rich floodplain meadows (Böger, 1991; Bissels et al., 2004). However, typical species hardly re-immigrated, and the re-established grassland in the study area remained rather species-poor (Schmiede et al., 2012). From 2005 to 2008, freshly mown plant material (seed-containing green hay *sensu* Kiehl et al., 2010) was gained from eight donor sites to create 20 strips (“restoration sites”) on different species-poor grassland sites. The donor sites consisted of species-rich *Molinion* or *Cnidion* meadows. Each restoration site was 120 m long and 10 m wide and divided in three segments of 40 m length, each of which had been prepared 2–7 weeks before the plant material transfer. All three segments had been mown, and then treated as following:

- *rotovated* twice,
- *ploughed* and harrowed, or
- left *untilled*.

Rotovating broke up the soil surface, while ploughing turned over the topsoil, with subsequent harrowing breaking up the new surface and levelling it (Supplementary Figure A1). Both treatments left a fine-grained seedbed with close to no intact vegetation, but elimination was more complete after ploughing and harrowing. However, depending on the timespan until plant material application, modest regrowth occurred on both treatments. Treatments were randomly arranged on each restoration site. Plant material transfer took place between mid-September and the end of October, when most species on the donor sites carried ripe seeds. Harvest coincided with the first cut (two donor sites, five restoration sites) or second cut (six donor sites, 15 restoration sites), depending on the mowing regime at the donor sites. A detailed description of restoration measures and sites can be found in Schmiede et al. (2012).

Vegetation sampling

Between 10 May and 13 June 2021, we investigated the vegetation on 254 plots (25 m^2). From these, 180 were located on the 20 restoration sites (nine per site, three per treatment). For nine of the 20 restoration sites, the plots had been previously studied by Schmiede et al. (2010) annually in the first 3 years after restoration, enabling comparison over time.

Additionally, as a reference, we placed 40 plots (two per restoration site) on the unrestored grassland surrounding all restoration sites, with a distance > 15 m to the restoration sites. Furthermore, on the eight donor sites, 34 plots were surveyed (3–5 per site, depending on their size). To enable comparability with the data of Schmiede et al. (2012), species abundance was recorded using the modified Braun-Blanquet scale (van der Maarel, 1979). For data analysis, species abundance classes were transformed to percentage values following the approach of Schmiede et al. (2012). In addition to the vegetation plots, we recorded whole-site species lists for restoration and donor sites and estimated species abundances using a DAFOR scale (Norfolk Wildlife Trust, n.d.) with modifications (Supplementary Table A1).

Soil and biomass sampling and analysis

In April and May 2021, we gathered soil samples from each of the 254 plots. To this end, composite samples of four topsoil cores (0–10 cm) were collected using a soil corer of 2.5 cm diameter. Samples were air-dried and sieved to 2 mm. Soil pH was measured in $CaCl_2$ solution. The samples were extracted with calcium-acetate-lactate solution (CAL) for the determination of plant-available potassium (K) and phosphorus (P) (Blume et al., 2000). Total soil nitrogen (N) and carbon (C) were measured *via* elementary analysis (device "Unicube," co. "elementar"; DIN EN 16168, 2012; DIN EN 15936, 2012), anorganic C was calculated from the $CaCO_3$ content determined with the Scheibler method (Blume et al., 2000). The organic C content was calculated as the difference between total and anorganic C content, and the C/N ratio as the ratio between organic C and total soil N content (Kuntze et al., 1994).

For each plot, aboveground biomass was harvested in four randomly placed quadrats of 0.1 m^2 at a height of 5 cm. Sampling took place between end of May and beginning of June, shortly before the regular first grassland cut at 8 June. Most of the donor sites are cut later in summer, but were sampled at the same time for comparability. Biomass samples were merged for each plot to one composite sample, dried at 60°C for 48 h, weighed, and milled to 0.5 mm. The acid detergent fibre (ADF), N, K, and P contents were determined *via* Near Infrared Spectroscopy (NIRS, details see Kleinebecker et al., 2011). As measures of nutrient stoichiometry, we calculated the N/K and N/P ratios. For feeding value assessment, we calculated the crude protein content (XP; Roth et al., 2011), the digestible energy (DE) for horses (National Research Council, 1999), the metabolisable energy (ME) for ruminants, and the net energy for lactation (Kirchgeßner and Kellner, 1982).

Hydrological variables

For calculation of hydrological variables, we used data from 33 groundwater wells (HLNUG, 2021) and daily water levels for 12 points of the river Rhine along the study area between 1 January 2001 and 31 December 2020. If data gaps for the groundwater wells were ≤ 30 days, we interpolated the groundwater levels (GWL) between adjacent time points to obtain daily groundwater water levels. The daily Rhine water levels were linearly interpolated between gauging stations Mainz, Nierstein-Oppenheim, and Worms (Wasserstraßen-und Schifffahrtsverwaltung des Bundes, 2021). The 45 groundwater points were used for daily Delaunay triangulation (Sinclair, 2016), including all points with an entry for the respective day. The daily groundwater level of each of the 254 plots was estimated as the inverse-distance weighted mean of the three nearest groundwater points. For each plot, we calculated three relevant hydrological predictors for species distribution (following Gattringer et al. (2019)):

- days per year with GWL > 0.7 m below ground ("Drought frequency").
- days per year with inundation height > 0.5 m ("Flood frequency").
- standard deviation of the GWL ("SD of GWL").

The location in the fossil floodplain, which is protected from flooding by a dyke, and the recent functional floodplain is often used as a hydrological predictor for ecological properties of floodplain meadows (e.g., Bissels et al., 2004; Donath et al., 2007). For our restoration sites, it was well represented by the SD of GWL. The mean was 0.72 m ± SD of 0.14 m for sites located in the functional floodplain, and 0.37 m ± 0.11 m for sites located in the fossil floodplain, respectively. Thus, we focused on the SD of GWL for further analysis, instead of the floodplain compartment.

Statistical analysis

To assess the impact of soil preparation over time, we compared the number and cover of target species (Schmiede et al., 2012, slightly modified, Table 1) as well as the number of species of the three soil preparation treatments for each of the first 3 years after restoration and for 2021 separately for the previously studied restoration sites. If an ANOVA indicated significant differences, these were identified with a Tukey honest-significant difference test (HSD; $\alpha = 0.05$). Data were ln-transformed to meet normality and homoscedasticity, and model assumptions were checked visually using diagnostic plots (Kozak and Piepho, 2018).

To assess temporal trends of the individual target species, we calculated their occurrence frequencies in the third year after restoration and in 2021 for the previously studied restoration sites on a plot basis. For comparison, occurrence frequencies in the plant material and mean diaspore input per target species for each restoration site were calculated from the plant material data of Schmiede et al. (2010). We performed analogous frequency calculations for the corresponding unrestored reference plots of the previously studied restoration sites and for the donor site plots from the 2021 data.

We explored the temporal development of the vegetation composition on the restoration sites using non-metric multidimensional scaling (NMDS) ordination for the previously studied restoration sites for (a) the first 3 years after restoration and (b) for 2021, including the corresponding unrestored reference and donor site plots. We performed a second NMDS for all restoration sites, unrestored reference plots, and donor sites. Ordinations were based on Bray-Curtis Distances, max. 100 iterations, and a random starting configuration. Three-dimensional solutions were chosen by visually checking the decrease of stress values with increasing number of dimensions, according to Leyer and Wesche (2008). To explore underlying ecological gradients, we included vectors for the number of species and target species, the proportions of plant life forms and life span groups, mean Ellenberg indicator values for light (L),

TABLE 1 Development of target species over time.

			Frequency (%)					
Target species	RL	Trend on RS	RS (third year)	RS (2021)	UG	DS	PM	MDI
Increased frequency								
Arabis hirsuta	V	↗	0	17	11	15	0	0
Bromus erectus	*	↗	0	6	0	32	22	6
Carex praecox	V	↗	2	15	6	12	78	12
Galium boreale	V	↗	9	17	0	15	78	225
Genista tinctoria	V	↗	2	11	0	41	22	9
Inula britannica	V	↗	9	11	0	6	89	1,636
Inula salicina	V	↗	17	26	11	53	100	890
Iris spuria	2	↗	0	21	0	12	33	9
Peucedanum officinale	3	↗	9	17	0	50	56	6
Pimpinella saxifraga	*	↗	4	5	0	6	33	6
Scutellaria hastifolia	2	↗	2	4	0	3	33	1
Viola pumila	2	↗	0	5	0	6	56	15
Viola stagnina	2	↗	1	4	0	12	11	5
Reduced frequency								
Arabis nemorensis	2	↘	47	31	11	12	89	1,294
Bupleurum falcatum	V	↘	4	0	0	3	0	0
Dipsacus laciniatus	*	↘	6	0	0	0	11	0
Linum catharticum	*	↘	14	1	6	12	78	39
Rhinanthus alectorolophus	*	↘	5	0	0	6	0	0
Selinum carvifolia	V	↘	6	2	11	18	44	16
Senecio aquaticus	V	↘	5	2	0	0	0	0
Silaum silaus	V	↘	7	5	6	21	33	4
Stable frequency								
Sanguisorba officinalis	V	↔	20	19	0	62	67	2
Thalictrum flavum	V	↔	4	4	6	9	33	2
Valeriana pratensis.	*	↔	15	15	11	21	11	1
Veronica maritima	V	↔	19	19	0	6	56	89
No establishment								
Allium angulosum	3	-	0	0	0	15	78	139
Betonica officinalis	V	-	0	1	0	12	0	0
Bromus racemosus	3	-	0	2	11	12	0	0
Carex panicea	V	-	0	0	0	18	22	1
Carex tomentosa	3	-	1	0	0	29	89	4
Cirsium tuberosum	3	-	2	1	6	15	0	0
Gentiana pneumonanthe	2	-	0	0	0	3	0	0
Hippocrepis comosa	V	-	1	0	0	6	0	0
Iris sibirica	3	-	0	1	0	12	11	0
Juncus alpinoarticulatus	V	-	0	0	0	0	0	0
Lathyrus palustris	3	-	0	0	0	3	0	0
Lotus maritimus	3	-	1	0	0	9	22	1
Lotus tenuis	V	-	2	0	0	3	33	3
Melampyrum cristatum	3	-	2	0	0	21	0	0
Molinia caerulea	*	-	2	0	0	24	56	40
Potentilla erecta	*	-	2	0	0	12	22	1
Sanguisorba minor	*	-	1	0	0	6	0	0
Selinum dubium	2	-	0	0	0	3	0	0
Serratula tinctoria	3	-	1	1	0	32	56	10
Succisa pratensis	V	-	1	0	0	32	11	0
Viola elatior	2	-	0	0	0	6	44	1

Red List status (RL) refers to Germany (Metzing et al., 2018). *, not endangered; V, warning list; 3, endangered; and 2, seriously endangered. For the previously studied restoration sites (RS), plot-level frequencies are given for the third year after restoration and for 2021 ($n = 81$, respectively). The trends are only given for species with a frequency > 3% in at least 1 year. If the change is ≤20% of the third year value, the trend is regarded as stable. Plot-level frequencies for the unrestored reference grassland (UG, $n = 18$) and the donor sites (DS, $n = 34$) refer to the 2021 vegetation surveys. Frequencies for plant material (PM) and the mean diaspore input (MDI, units per m^2) refer to plant material samples ($n = 9$) taken by Schmiede et al. (2010).

temperature (T), continentality (K), moisture (F), nutrients (N), and soil reaction (R), the proportion of indicator species for alternating water levels (data from Klotz et al., 2002), as well as the soil, biomass, and hydrological variables described above. The package "vegan" was used for the ordination (Oksanen et al., 2020).

We compared the different soil preparation treatments of all restoration sites with the unrestored reference grassland and the donor sites concerning soil C/N ratio, total soil N, plant available soil P and K, species and target species numbers, cover of target species, biomass yield, and energy content measures. Variables were pooled at the treatment level for the restoration sites and at the site level for the references. To this end, we performed an ANOVA, followed by a Tukey HSD test ($\alpha = 0.05$). Data were ln-transformed if diagnostic plots indicated violations against model presumptions (Kozak and Piepho, 2018).

We calculated four indicators to quantify the long-term restoration success of the restoration sites at the site level, following Kiehl et al. (2010) (Supplementary Table A2). These were (a) absolute transfer rate of species, (b) absolute transfer rate of target species, (c) relative transfer rate of species, and (d) relative transfer rate of target species. We defined absolute and relative transfer rates for all species and for target species as the ratios between transferred and transferable species. For absolute transfer rates, species were regarded as transferable if their DAFOR abundance was R2 or higher on at least one corresponding donor site of a restoration site. For relative transfer rates, species found in the plant material from the respective restoration site (Schmiede et al., 2010) were regarded as transferable. A transferable species was regarded as transferred if recorded on a restoration site in 2021. Species from the corresponding unrestored reference plots were regarded as resident and excluded from the pool of transferable and transferred species for the respective restoration site. We opted for the calculation of both absolute and relative transfer rates as we had more data points for the absolute transfer rates ($n = 20$). However, since the relative transfer rates ($n = 15$) are based on the species composition of the plant material used for restoration, they are considered a more direct success measure. At plot level, we calculated the (e) increase in target species number and (f) increase in target species cover as the difference between the plot on the restoration site and the mean of the corresponding unrestored reference plots.

To identify factors determining the restoration success, linear regression models were used for the six success variables (a–f) separately at site level. Eleven explanatory variables were included in the model selection using the "dredge" function (R package MuMIn, Bartoń, 2020). These were the N/P and N/K ratio of the biomass, biomass yield, soil pH, plant-available P and K, soil organic C content, and the C/N ratio. Soil N content was not included due to high correlation with soil organic C ($r = 0.995$). Drought and flood frequency and the SD of GWL were included as hydrological variables. The explanatory variables were centred to a mean of 0 and scaled to a standard deviation of 1. The model with the lowest AIC that showed no multicollinearity (all variance inflation factors ≤ 2.5) was selected.

Results

Development of the restoration sites over time

The development over time can only be assessed for the previously studied restoration sites, which were restored in 2005 and 2006, so the observation period is 15–16 years here. After that time, the differences between soil preparation treatments concerning target species number and cover vanished (Figure 1). Compared with the first years after restoration, in the long-term, both variables remained relatively stable for the ploughed and rotovated treatments but increased for the untilled treatment. In 2021, the mean number of target species per 25 m^2 ranged from 2.2 ± 0.5 (ploughed, mean ± SE) to 3.0 ± 0.5 (rotovated), and mean target species cover ranged from 3.1 ± 0.8 (untilled) to $3.5 \pm 0.9\%$ (rotovated). The mean number of species per plot was around 25 for all treatments. For the untilled treatment, this marked a stable trend since the third year after restoration, whereas the species number per plot decreased from around 38 to 25 species for the treatments with soil preparation. This finding was supported by the NMDS ordination of the previously studied restoration sites indicating that species composition of soil disturbance plots became more similar to the unrestored reference plots until 2021 (Supplementary Figure A2).

Out of the 46 target species, 13 showed a higher frequency at the restoration sites in 2021 compared to 3 years after restoration (Table 1). Among these, we found a range of Red List species, such as *Carex praecox*, *Galium boreale*, *Genista tinctoria*, *Iris spuria*, and *Peucedanum officinale*. During the investigation period, eight target species decreased in frequency. These had been mostly recorded with low frequencies by Schmiede et al. (2010) already, such as *Bupleurum falcatum*, *Rhinanthus alectorolophus*, and *Selinum carvifolia*. An exception was *Linum catharthicum*, which was recorded only in 1% of the restoration plots in 2021, compared to 14% 3 years after restoration. *Sanguisorba officinalis* and *Veronica maritima* remained relatively stable with a frequency of around 20%, respectively.

Ecological comparison between restoration sites and references

The NMDS of all restoration sites and the references for 2021 revealed that the donor sites were separated from the unrestored reference grassland and the restoration sites (Figure 2). While there was a wide overlap between the latter two groups, the centroid of the unrestored reference grassland was separated from the centroids of the restoration soil treatments, which were all slightly shifted towards the donor sites. The donor sites were characterised by higher target species and species numbers, energy contents, Ellenberg R values, and drought frequency compared to the other groups. Both the restored and unrestored sites were characterised by higher productivity levels, indicated by

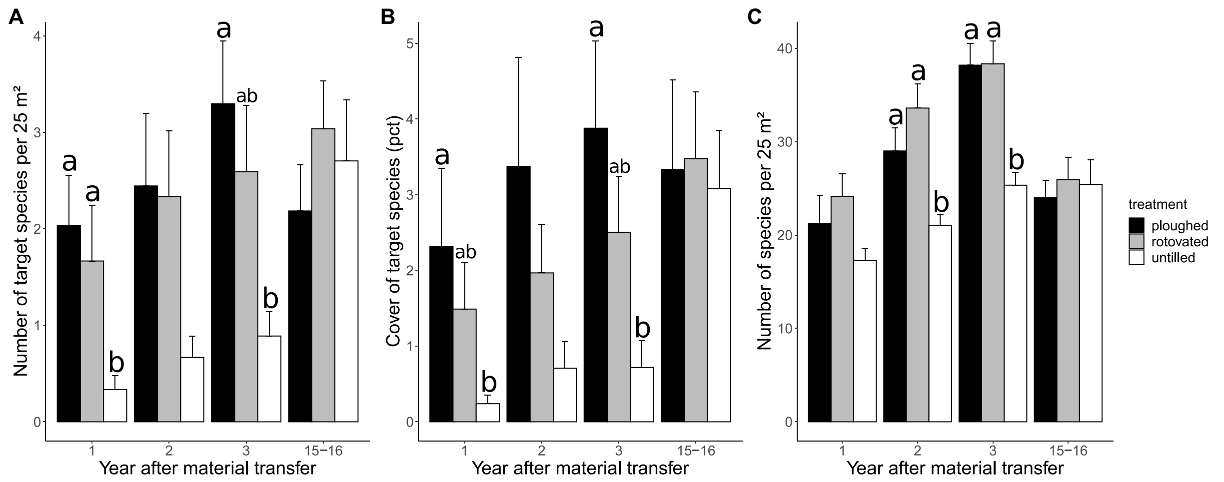


FIGURE 1
Development of the number **(A)** and cover **(B)** of target species and the number of species **(C)** per 25m² over time after the transfer of plant material on the previously studied restoration sites for the three different treatments ploughed, rotovated, and untilled (*n*=9, respectively). Significant differences within years (*p*<0.05, ln-transformed data) are indicated by different letters above the bars. Whiskers refer to the standard errors.

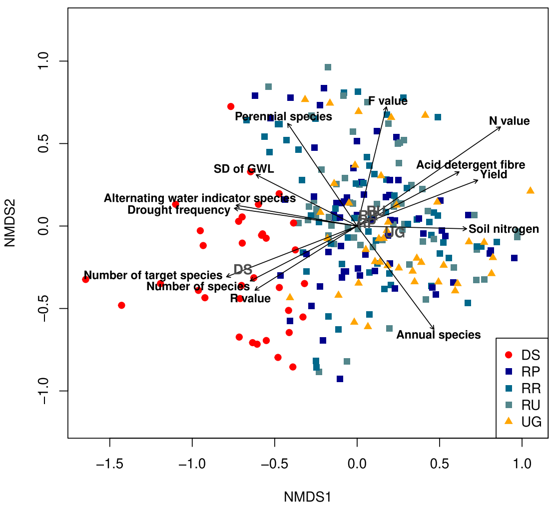


FIGURE 2
Non-metric multidimensional scaling (NMDS) of the vegetation plots for all restoration sites, the unrestored reference grassland and the donor sites in 2021 (axis 1 and 2 of the three-dimensional solution are shown). Final stress: 16.9. Plots are grouped by donor sites (DS), unrestored reference grassland (UG), and restoration sites with the treatments ploughed (RP), rotovated (RR), and untilled (RU). The group labels are located at the centroids of the groups. Vectors with r^2>0.3 are displayed. For better readability, the cover of target species, the metabolisable energy (high correlation with number of species and target species) were removed despite r^2>0.3.

increased Ellenberg N values and biomass yields compared with the donor sites.

Concerning soil nutrient status, the restoration sites and the unrestored reference grassland were very similar (Figure 3). Their C/N ratio averaged at 11.0 ± 0.1 (± SE), compared to 11.9 ± 0.3 for the donor sites. No significant differences between the groups were found for total nitrogen, plant-available P and K contents. However, soil nutrient contents of the donor sites were lower than those of the restoration sites and unrestored reference plots. Especially the low and very narrow plant-available P content of the donor sites (0.9 ± 0.1%) was noticeable.

In 2021, over all restoration sites, the number of target species per plot was similar for all soil treatments with an average of 1.9 ± 0.3 (Figure 4A) and significantly higher than for the unrestored reference grassland (0.7 ± 0.2), but significantly lower than for the donor sites (7.0 ± 1.4). Although not significant, mean cover of target species was higher on the restoration sites (2.1 ± 0.4%) than on the unrestored reference grassland (1.3 ± 0.6%; Figure 4B). For the donor sites, however, target species cover was significantly and markedly higher (mean = 19.7 ± 5.0%). The same held true for the number of recorded plant species (Figure 4C).

Feeding value of the grassland stands

The biomass yield levels of the restoration sites (407 ± 26 g/m²) and the unrestored reference grassland (421 ± 36 g/m²) did not differ but both were significantly higher compared to the donor sites, which had an average yield of 239 ± 29 g/m² (Figure 5A). The energy content variables for cattle and horses were similar between restoration sites and the unrestored reference grassland, with those of the donor sites being 4–5% higher (Figures 5B–D).

Drivers of restoration success

Absolute transfer rates of both all species and target species averaged at 40.1 ± 3.9 and 36.4 ± 6.2%, respectively

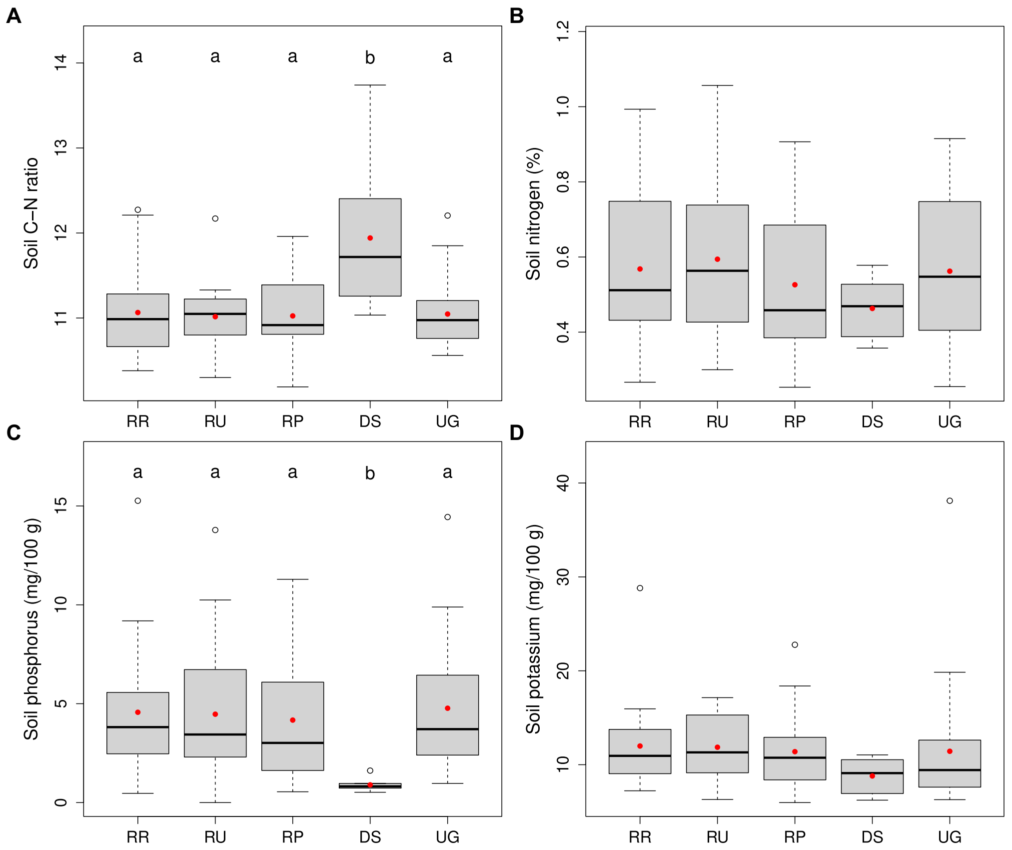


FIGURE 3
Box-whisker plots of the soil C/N ratio **(A)**, total nitrogen (%; **B**), plant-available P (mg/100g; **C**), and plant-available K (mg/100g; **D**) in 2021 on the different site categories—donor sites (DS, *n*=8), all restoration sites with treatments ploughed (RP), rotovated (RR), and untilled (RU), and unrestored reference grassland (UG; *n*=20, respectively). Plot data were averaged on the treatment level or, in case of DS and UG, on the site level. Red dots display the mean values. Significant differences ($p<0.05$) are indicated by different letters above the boxes (testing on ln-transformed data for soil P and soil K).

(Supplementary Table A2). The corresponding relative transfer rates were 24.9 ± 1.6 and 34.7 ± 3.7%. Higher biomass yield was generally associated with lower numbers of target species and reduced absolute transfer rates of species and target species (Table 2). The C/N ratio was positively associated with both the number and the cover of target species. Out of the hydrological variables, higher flood frequency tended to reduce restoration success, while higher drought frequency and variation of the groundwater level tended to have positive effects. The R^2 of the selected models ranged from 36 to 65%, except for the relative transfer rate of species, which could not be explained ($R^2 = 1\%$).

Discussion

Vegetation development over time

We found no effect of soil preparation on vegetation development and target species establishment 13–16 years after restoration. This is surprising, as one of the main findings of Schmiede et al. (2012) was that soil disturbance, especially ploughing, enabled better (target) species establishment due to suppression of the existing grassland vegetation. This is a common observation among different grassland types, so that soil preparation prior to diaspore introduction in species-poor grassland is often recommended (Kiehl et al., 2010). However, studies deriving such advice from their findings mostly have short observation timeframes and to the best of our knowledge do not exceed 8 years (Edwards et al., 2007; Bischoff et al., 2018; Durbecq et al., 2021). In line with our findings, recent studies on floodplain meadow restoration by Harvolk-Schöning et al. (2020) and Heilscher (2020) indicate that the positive effect of soil preparation on the number and cover of introduced species diminishes in the long run.

Initially, soil disturbance creates micro-niches for germination and establishment of species from the plant material (Harvolk-Schöning et al., 2020), but also activates the soil seed bank (Schmiede et al., 2012; Ludewig et al., 2021). In the short term, this leads to promotion of ruderal species (Klaus et al., 2018). Accordingly, in our experiment, ruderal species, such as *Cirsium arvense*, *Galium aparine*, or *Lactuca serriola* emerged in high frequencies over the first 3 years after restoration, but receded in

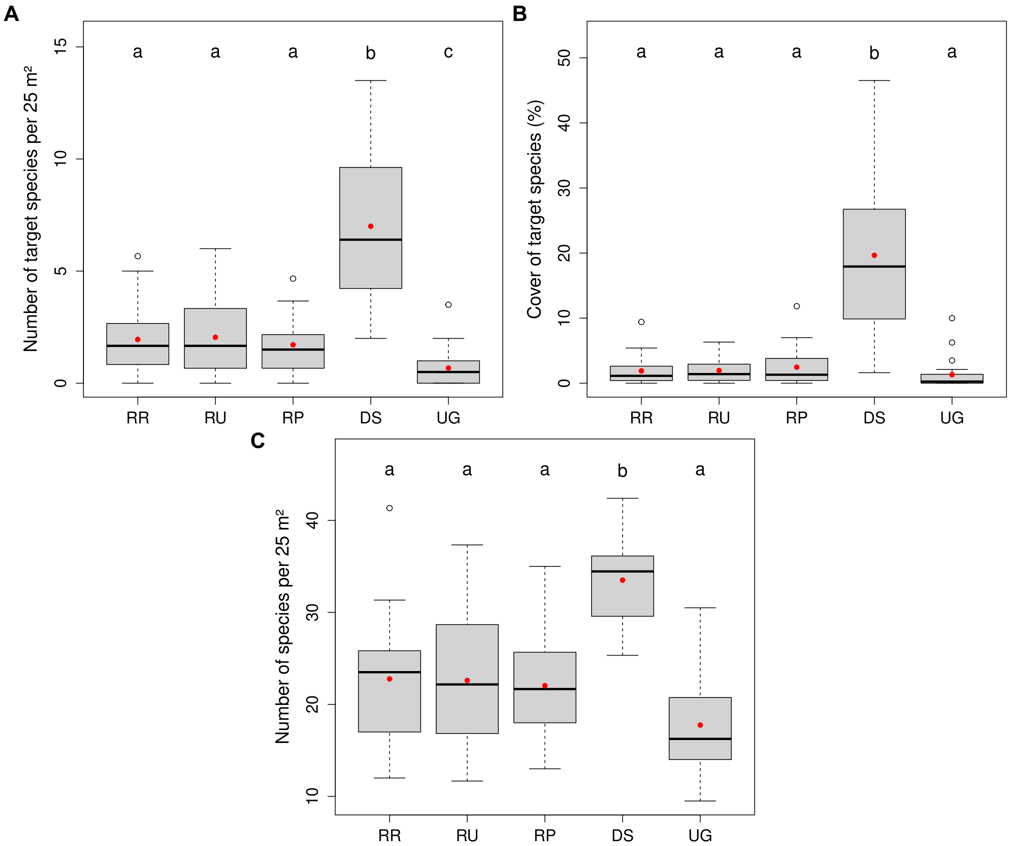


FIGURE 4
Box-whisker plots of the number of target species per plot **(A)**, the cover of target species (%; **B**), and the number of species per 25m^2 plot **(C)** in 2021 on the different site categories—donor sites (DS, *n*=8), all restoration sites with treatments ploughed (RP), rotovated (RR), and untilled (RU), and unrestored reference grassland (UG; *n*=20, respectively). Plot data were averaged on the treatment level or, in case of DS and UG, on the site level. Red dots display the mean values. Significant differences ($p<0.05$) are indicated by different letters above the boxes (testing on ln-transformed data for number and cover of target species).

the long run. In contrast, some target species such as *Inula britannica* or *Carex praecox* emerged later or developed more slowly. This was presumably due to competition with the resident vegetation, but these species established in the long run even without tillage.

Across all treatments, target species that were already present 3 years after restoration mostly remained stable or increased in frequency until 2021. This is in accordance with the stable target species number on plots with soil disturbance and the observed increase for the untilled treatment. An especially encouraging case is *Iris spuria*, which was not detected by Schmiede et al. (2012) in the first 3 years after restoration, but was detected in considerable amounts on two restoration sites in 2021. The hard seed coat of this species can delay germination, so that establishment happens only after longer time periods (Hölzel and Otte, 2004b). Harvolk-Schöning et al. (2020) observed a similar pattern for *Iris spuria* on former arable fields. Our results clearly show that in the longer term, the establishment of this highly endangered species is possible on grasslands lacking typical floodplain meadow species.

Many target species were not successfully established, some of them despite frequent occurrence on the donor sites. For example, *Succisa pratensis* was barely captured in the plant material, which may be due to asynchronous fruit ripening with only a small proportion of ripe seeds when the plant material was harvested, as this species has a long flowering and seed shedding period (Adams, 1955). However, after-ripening of seeds may lead to increased germination even when they are harvested in an unripe state, as was shown for the non-native *L. polyphyllus* in mountain grasslands (Klinger et al., 2020). Diaspores of *Allium angulosum*, *Selinum carvifolia*, and *Serratula tinctoria* were captured in considerable amounts (in ≥44% of plant material samples and with ≥10 diaspores per m^2 on average, respectively), but established poorly or not at all, with an occurrence frequency of 6% at maximum on the previously studied restoration sites over the whole observation period. This matches with observations by Harvolk-Schöning et al. (2020), and could be a consequence of specific germination requirements, e.g., characteristic temperature regimes (Hölzel and Otte, 2004a; Wagner et al., 2021).

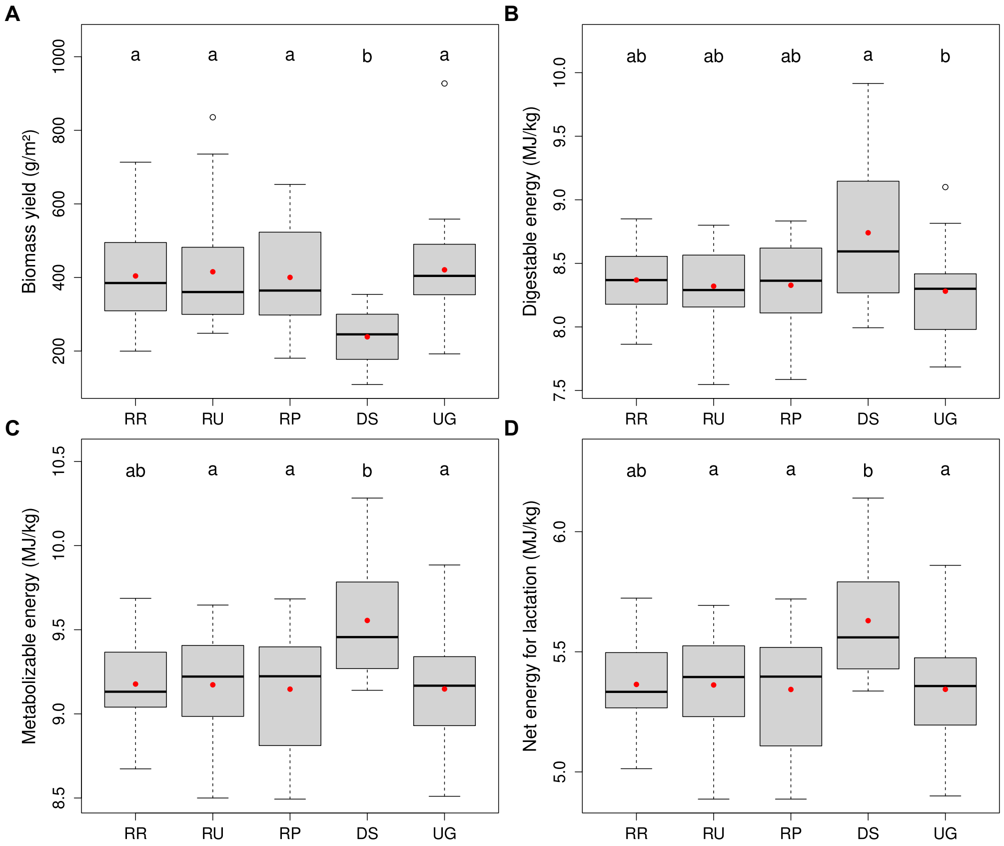


FIGURE 5
Box-whisker plots of the biomass yield (g/m^2; **A**), the digestible energy for horses (MJ/kg; **B**), the metabolisable energy for ruminants (MJ/kg; **C**), and the net energy for lactation (MJ/kg; **D**; all referring to dry matter) in 2021 on the different site categories—donor sites (DS, *n*=8), all restoration sites with treatments ploughed (RP), rotovated (RR), and untilled (RU), and unrestored reference grassland (UG; *n*=20, respectively). Plot data were averaged on the treatment level or, in case of DS and UG, on the site level. Red dots display the mean values. Significant differences ($p<0.05$) are indicated by different letters above the boxes (testing on ln-transformed data for biomass yield).

Ecological comparison of restoration sites, unrestored reference grassland, and donor sites

Our findings on the nutrient levels for the grassland sites overall matched those of former studies in the region (Donath et al., 2007, 2015; Schmiede et al., 2012). Grasslands of high nature conservation value, often used as donor sites, consistently had much lower plant-available P contents and moderately lower plant-available K contents than species-poor grassland sites often chosen for restoration. For the N contents, no such pattern had been found in those studies. In our study, the tendency to increased N contents of the restoration sites and the untreated reference grasslands compared with the donor sites is probably due to seven restoration sites with high organic C contents of 8.6 ± 0.5% (mean ± standard error; vs. 4.9 ± 0.3% for the other 13 restoration sites). The higher average C/N ratios of the donor sites are mainly driven by two poor *Molinion* sites with very wide ratios of 12.9 and 13.7, respectively.

A range of rare and endangered plant species of floodplain meadows, many of which are listed in the Red Lists of Germany and Hesse, were successfully established on the restoration sites. However, our results confirm that the ecological restoration of grassland is challenging, even if the sward is disturbed prior to diaspore introduction (Kiehl et al., 2010; Harvolk-Schöning et al., 2020; Hansen et al., 2022). Thirteen to sixteen years after restoration, the vegetation composition of the restored plots was similar to the unrestored reference grassland plots, with only slight changes towards the composition of the donor sites. Nevertheless, the number of target species was significantly higher for restoration sites and also their cover increased, compared with the unrestored reference.

Feeding value of the site categories

Different restoration measures affected neither the yield nor the energy content of the aboveground biomass. While a change in yield was not expected, a more diverse species composition

TABLE 2 Overview of the regression models selected by AIC criterion for the ecological restoration success variables on the site level.

	Absolute TR target species (%)	Absolute TR species (%)	Relative TR target species (%)	Relative TR species (%)	Increase in target species number per plot	Increase in target species cover (%)
Intercept	5.578***	40.111***	21.993**	24.952***	1.231***	28.588***
Biomass yield	−1.564***	−9.817*	.	.	−0.591*	.
Soil C/N ratio	.	.	.	.	1.039***	4.657*
Soil P	.	5.968	.	.	.	.
Soil pH	.	.	15.183	.	.	.
SD of GWL	.	.	.	.	.	4.925*
Flood frequency	−0.998*	−4.351	.	−1.784	−0.567*	−2.682
Drought frequency	.	.	13.310*	.	.	.
n	20	20	15	15	20	20
R^2	0.65	0.39	0.36	0.01	0.60	0.42
AIC	78.2	166.4	120.2	100.2	60.4	145.9
λ	0.5	1	1	1	1	1.5

TR, transfer rate. Rows represent the estimates for the different explanatory variables included in the selected models—the biomass yield, the C/N ratio, plant-available P content and pH of the soil, the SD of the groundwater level, the flood and drought frequency (all standardised to mean = 0 and SD = 1). Variables with a dot were not selected in the respective model. Significance levels are given as following: ***$p \leq 0.001$, **$p \leq 0.01$, and *$p \leq 0.05$. *n*, number of observations; R^2, adjusted R^2 of the model; AIC, akaike information criterion; and λ, value of λ for the Box-Cox transformation of the response variable.

with a higher proportion of forbs can be associated with higher energy contents of the biomass in floodplain meadows (Donath et al., 2004). The similar biomass energy contents of restored and unrestored reference grasslands can be explained by the marginal effects of restoration measures on the overall vegetation composition.

The yield levels of the restored and unrestored grasslands in our study system are mostly within the previously observed range of non-intensively managed grasslands of wet and mesotrophic sites (Tallowin and Jefferson, 1999; Donath et al., 2015). With dry matter yields of up to 705 g/m^2, some sites exceeded the levels normally reached without fertilisation (Tallowin and Jefferson, 1999). Under these conditions and with regard to the current subsidy policy (EU area bonus and conservation contracts), haymaking is economically viable for the regional farmers. For lactating cows, the hay may be at best recommended as basic feed, as the net energy for lactation of 5.4 ± 0.2 MJ/kg dry matter (mean ± SD) would require supplementation with high-energy compounds (Donath et al., 2004, 2021; Schumacher, 2016). The metabolisable energy contents of 9.2 ± 0.3 MJ/kg dry matter indicate suitability as complete feed for non-lactating cows (Donath et al., 2004; Deutsche Landwirtschafts-Gesellschaft, 2009) and empty ewes or ewes in early pregnancy, as well as for integration in compound feed rations for calves (Bayerische Landesanstalt für Landwirtschaft (LfL), 2021). Practically, most of the hay harvested in the region is used for leisure horses. The hay from our restoration sites and their surroundings is suitable for this with regard to the observed digestible energy levels of 8.3 ± 0.3 MJ/kg dry matter (National Research Council, 1999; Donath et al., 2004).

Slightly higher energy contents of the biomass from highly species-diverse donor sites indicate that an increase in species diversity does not preclude the integration of species-rich swards into feeding rations (Tallowin and Jefferson, 1999; Donath et al., 2004). However, yield of the donor sites is on average 40% lower compared to the restoration sites, which makes it difficult for farmers to operate profitably. Thus, agri-environmental schemes obviously remain an important pre-requisite in the conservation of species-rich grasslands of high-nature value (Donath et al., 2021).

Drivers of restoration success

The transfer rates we observed were within the typical range for plant material transfer on species-poor grassland, but lower than on former arable fields (Kiehl et al., 2010). This holds true for both target species as well as total species numbers. While biomass yield and flood frequency had negative impacts, wider C/N ratios positively affected restoration success. These three predictors were identified as significant for at least two success variables.

Biomass yield levels are the result of complex interactions of biotic and abiotic factors (Doyle, 1982), with different nutrients being decisive in different locations and years (Fay et al., 2015). Beside generally relatively fertile soils in floodplains, we suspect that productivity in our restoration sites is partly increased by remnants of former fertilisation, which may be the reason for the very high yield levels of some of the sites. Due to the dominance of tall and highly productive grasses under fertile conditions (Honsova et al., 2007), high productivity reduced the suitability for the establishment of species-rich floodplain meadows.

Regular flooding events lead to nutrient deposition in close proximity to the river channel (Klaus et al., 2011; Poulsen et al., 2014) and increase the productivity of floodplain meadows by higher soil nutrient levels (Beltman et al., 2007). Apart from this, higher water availability increases mineralization and nutrient supply, which leads to highly variable biomass yield between years (Mathar et al., 2015), but also between sites (*cf.* Jakrlová, 1999). In our study, the positive relationship between flood frequency and biomass yield ($r = 0.35$, Supplementary Figure A3) could result from a mixture of the fertilising and the mineralizing effect of frequent flooding events. This may explain the identification of flood frequency as negatively affecting restoration success along with the biomass yield observed in 2021. Another reason for the adverse effect of flood frequency on restoration success might be that long flooding of seedlings emerged from transferred plant material impedes survival (Bao et al., 2018; Gattringer et al., 2018).

Increased soil C/N ratios were significantly associated with increases in target species number and cover. The C/N ratio in the soil as an indicator of N availability could be another long-term determinant of productivity. However, the positive correlation between C/N ratio and yield ($r = 0.30$) does not support this. Higher C/N ratios, although not reducing productivity, could facilitate the establishment of typical floodplain meadow species by reducing competition with generalist grassland species adapted to high and continuous N availability. Accordingly, adverse effects of soil nitrogen on target species establishment were observed in the floodplain of the river Elbe (Dullau et al., 2021).

While Schmiede et al. (2012) identified plant available P content in the soil to negatively affect target species numbers in our study system, our resurvey cannot confirm this for the long term. Considering an extended set of factors and sites, the restoration sites rich in organic C, which had not been covered by the study of Schmiede et al. (2012), were among the more productive ones, so that the overall effect of biomass yield might have masked the effect of soil P.

Conclusion

In our study, we found no long-term effect of soil preparation on vegetation development and target species establishment across a large dataset. This indicates that the positive effect of soil preparation on the number and cover of target species, which is regularly reported in short-term studies, diminishes over time, while the effects of local site conditions become more important. Therefore, soil preparation prior to seed introduction may not be necessary in floodplain meadow restoration. To increase restoration success, the productivity of restoration sites, soil C/N ratios, and flooding frequency should fit to the respective restoration goals. For practitioners, choosing restoration sites with productivity levels not greatly exceeding those of the donor sites may be most feasible. If restoration sites are too productive, management schemes that actively reduce site productivity are recommended.

Concerning biomass characteristics, we showed that despite considerable differences in yield, even restoration sites with low productivity provide biomass of sufficient amount and feeding value. Thus, the integration of restored grasslands in local farming systems is possible and can ensure long-term management and thus stability of these ecosystems. Furthermore, one-time introduction of target species showed only limited success. Thus, additional plant material transfer or manual seeding of target species is probably necessary. Further studies should investigate the potential of such supplementary measures. Overall, we strongly recommend long-term monitoring of restoration projects in other regions and grassland types, as factors determining restoration success may become evident only after longer time periods.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TWD, TK, and SH-S conceived of the research idea and designed the study. LS gathered and analysed the data with the help of YPK and SH-S, and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was funded by the German Federal Environmental Foundation (grant number 35171/01).

Acknowledgments

We thank the German Federal Environmental Foundation for funding the study. We express our gratitude to J. Scholz vom Hofe for his exertion in soil and biomass sampling and sample processing, to J. Höhl for the lab analyses, and to S. Flecken for her input on the feeding value aspects of this manuscript. The authors thank M. Harnisch from the city of Riedstadt and R. Baumgärtel from the forestry administration Groß-Gerau for their professional and administrative help at the Northern Upper Rhine, as well as to the local farmers who patiently supported our investigations and are grateful to N. Hölzel from the University of Münster for his ideas on the sampling design and the interpretation of results, and to M. Hahn and K. Willkomm for their help during field work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.1061484/full#supplementary-material

References

Adams, A. W. (1955). Succisa Pratensis Moench. *J. Ecol.* 43:709. doi: 10.2307/2257031

Bakker, J. P. (1989). *Nature Management by Grazing and Cutting.* Dordrecht: Kluwer academic publishers.

Bao, F., Elsey-Quirk, T., de Assis, M. A., Arruda, R., and Pott, A. (2018). Seasonal flooding, topography, and organic debris interact to influence the emergence and distribution of seedlings in a tropical grassland. *Biotropica* 50, 616–624. doi: 10.1111/btp.12550

Bardgett, R. D., Bullock, J. M., Lavorel, S., Manning, P., Schaffner, U., Ostle, N., et al. (2021). Combatting global grassland degradation. *Nat. Rev. Earth Environ.* 2:10. doi: 10.1038/s43017-021-00207-2

Bartoń, K. (2020). MuMIn: Multi-Model Inference (1.43.17). Available at: https://CRAN.R-project.org/package=MuMIn

Bayerische Landesanstalt für Landwirtschaft (LfL) (2021). *Gruber Tabelle zur Fütterung der Milchkühe, Zuchtrinder, Schafe, Ziegen*. 47th Edn.

Bekker, R. M., Verweij, G. L., Bakker, J. P., and Fresco, L. F. M. (2000). Soil seed bank dynamics in hayfield succession. *J. Ecol.* 88, 594–607. doi: 10.1046/j.1365-2745.2000.00485.x

Beltman, B., Willems, J. H., and Güsewell, S. (2007). Flood events overrule fertiliser effects on biomass production and species richness in riverine grasslands. *J. Veg. Sci.* 18, 625–634. doi: 10.1111/j.1654-1103.2007.tb02576.x

Bischoff, A., Hoboy, S., Winter, N., and Warthemann, G. (2018). Hay and seed transfer to re-establish rare grassland species and communities: how important are date and soil preparation? *Biol. Conserv.* 221, 182–189. doi: 10.1016/j.biocon.2018.02.033

Bissels, S., Hölzel, N., Donath, T. W., and Otte, A. (2004). Evaluation of restoration success in alluvial grasslands under contrasting flooding regimes. *Biol. Conserv.* 118, 641–650. doi: 10.1016/j.biocon.2003.10.013

Blume, H. P., Deller, B., Leschber, R., Paetz, K., Furtmann, K., and Wilke, B. M. (2000). *Handbuch der Bodenuntersuchung*. Berlin: Beuth Verlag.

Böger, K. (1991). Grünlandvegetation im Hessischen Ried. 286.

Burmeier, S., Eckstein, R. L., Otte, A., and Donath, T. W. (2010). Desiccation cracks act as natural seed traps in flood-meadow systems. *Plant Soil* 333, 351–364. doi: 10.1007/s11104-010-0350-1

Deutsche Landwirtschafts-Gesellschaft (2009). Empfehlungen zur Fütterung von Mutterkühen und deren Nachzucht. Available at: https://llg.sachsen-anhalt.de/fileadmin/Bibliothek/Politik_und_Verwaltung/MLU/LLFG/Dokumente/04_themen/fleischrind/10_fu-empfehl_mutterkuehe_nachzucht.pdf

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Agard, J., Arneth, A., et al. (2019). Pervasive human-driven decline of life on earth points to the need for transformative change. *Science* 366:eaax3100. doi: 10.1126/science.aax3100

DIN EN 15936 (2012). Schlamm, behandelter Bioabfall, Boden und Abfall_-Bestimmung des gesamten organischen Kohlenstoffs (TOC) mittels trockener Verbrennung; Deutsche Fassung EN_15936: 2012. Beuth Verlag GmbH.

DIN EN 16168 (2012). Schlamm, behandelter Bioabfall und Boden_-Bestimmung des Gesamt-Stickstoffgehalts mittels trockener Verbrennung; Deutsche Fassung EN_16168: 2012. Beuth Verlag GmbH.

Donath, T. W., Bissels, S., Hölzel, N., and Otte, A. (2007). Large scale application of diaspore transfer with plant material in restoration practice—impact of seed and microsite limitation. *Biol. Conserv.* 138, 224–234. doi: 10.1016/j.biocon.2007.04.020

Donath, T. W., Daniel, V., and Schneider, S. (2021). Long-term development of fodder quantity and quality of non-intensively managed grasslands in South-Western Luxembourg [PDF].

Donath, T. W., Hölzel, N., Bissels, S., and Otte, A. (2004). Perspectives for incorporating biomass from non-intensively managed temperate flood-meadows into farming systems. *Agric. Ecosyst. Environ.* 104, 439–451. doi: 10.1016/j.agee.2004.01.039

Donath, T. W., Holzel, N., and Otte, A. (2003). The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Appl. Veg. Sci.* 6, 13–22. doi: 10.1111/j.1654-109X.2003.tb00560.x

Donath, T. W., Schmiede, R., and Otte, A. (2015). Alluvial grasslands along the northern upper Rhine – nature conservation value vs. agricultural value under non-intensive management. *Agric. Ecosyst. Environ.* 200, 102–109. doi: 10.1016/j.agee.2014.11.004

Doyle, C. J. (1982). Modelling the determinants of grassland stocking rates on dairy farms in England and Wales. *Agric. Syst.* 9, 83–95. doi: 10.1016/0308-521X(82)90024-5

Dullau, S., Rydgren, K., Kirmer, A., Jäger, U. G., Meyer, M. H., and Tischew, S. (2021). The Dessau grassland experiment—impact of fertilization on forage quality and species assembly in a species-rich alluvial meadow. *Agriculture* 11:4. doi: 10.3390/agriculture11040339

Durbecq, A., d'Ambly, M., Buisson, E., Jaunatre, R., Cluchier, A., and Bischoff, A. (2021). Seedling recruitment in mountain grassland restoration: effects of soil preparation and grazing. *Appl. Veg. Sci.* 24, 1–11. doi: 10.1111/avsc.12564

Edwards, A. R., Mortimer, S. R., Lawson, C. S., Westbury, D. B., Harris, S. J., Woodcock, B. A., et al. (2007). Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. *Biol. Conserv.* 134, 372–382. doi: 10.1016/j.biocon.2006.08.025

Engst, K., Baasch, A., Erfmeier, A., Jandt, U., May, K., Schmiede, R., et al. (2016). Functional community ecology meets restoration ecology: assessing the restoration success of alluvial floodplain meadows with functional traits. *J. Appl. Ecol.* 53, 751–764. doi: 10.1111/1365-2664.12623

European Environment Agency (EEA) (2022). Term: Agri-environment scheme. Available at: https://www.eea.europa.eu/help/glossary/eea-glossary/agri-environment-scheme

Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., et al. (2015). Grassland productivity limited by multiple nutrients. *Nat. Plants* 1, 15080–15085. doi: 10.1038/nplants.2015.80

Freitag, M., Klaus, V. H., Bolliger, R., Hamer, U., Kleinebecker, T., Prati, D., et al. (2021). Restoration of plant diversity in permanent grassland by seeding: assessing the limiting factors along land-use gradients. *J. Appl. Ecol.* 58, 1681–1692. doi: 10.1111/1365-2664.13883

Gattringer, J. P., Ludewig, K., Harvolk-Schöning, S., Donath, T. W., and Otte, A. (2018). Interaction between depth and duration matters: flooding tolerance of 12 floodplain meadow species. *Plant Ecol.* 219, 973–984. doi: 10.1007/s11258-018-0850-2

Gattringer, J. P., Maier, N., Breuer, L., Otte, A., Donath, T. W., Kraft, P., et al. (2019). Modelling of rare flood meadow species distribution by a combined habitat surface water–groundwater model. *Ecohydrology* 12, 1–12. doi: 10.1002/eco.2122

Gough, M. W., and Marrs, R. H. (1990). A comparison of soil fertility between semi-natural and agricultural plant communities: implications for the creations of species-rich grassland on abondoned agricultural land. *Biol. Conserv.* 51, 83–96. doi: 10.1016/0006-3207(90)90104-W

Hansen, W., Klinger, Y. P., Otte, A., Eckstein, R. L., and Ludewig, K. (2022). Constraints in the restoration of mountain meadows invaded by the legume Lupinus polyphyllus. *Restor. Ecol.* 30:e13682. doi: 10.1111/rec.13682

Harnisch, M., Otte, A., Schmiede, R., and Donath, T. W. (2014). *Verwendung von Mahdgut zur Renaturierung von Auengrünland: 10 Tabellen*. Stuttgart: Ulmer.

Harvolk-Schöning, S., Michalska-Hejduk, D., Harnisch, M., Otte, A., and Donath, T. W. (2020). Floodplain meadow restoration revisited: long-term success of large scale application of diaspore transfer with plant material in restoration practice. *Biol. Conserv.* 241:108322. doi: 10.1016/j.biocon.2019.108322

Heilscher, C. (2020). Determinants of long-term restoration success in floodplain meadows. Master thesis, University of Münster, Germany.

Hejcman, M., Hejcmanová, P., Pavlů, V., and Beneš, J. (2013). Origin and history of grasslands in Central Europe—a review. *Grass Forage Sci.* 68, 345–363. doi: 10.1111/gfs.12066

HLNUG (2021). GruSchu—Hessen. GruSchu Hessen. Available at: https://gruschu.hessen.de/mapapps/resources/apps/gruschu/index.html?lang=de

HLNUG (2022). Wetterextreme. Wetterextreme. Available at: https://klimaportal.hlnug.de/wetterextreme

Hölzel, N. (1999). Flora und Vegetation der Auenwiesen im NSG Lampertheimer Altrhein—Eine aktuelle Zustandsanalyse mit Hinweisen zur zukünftigen Pflege und Entwicklung. *Jahrbuch Naturschutz Hessen* 4, 24–42.

Hölzel, N. (2019). "Limitierende Faktoren der Renaturierung," in *Renaturierungsökologie*. eds. J. Kollmann, A. Kirmer, S. Tischew, N. Hölzel and K. Kiehl (Berlin and Heidelberg: Springer), 35–52.

Hölzel, N., and Otte, A. (2003). Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. *Appl. Veg. Sci.* 6, 131–140. doi: 10.1111/j.1654-109X.2003.tb00573.x

Hölzel, N., and Otte, A. (2004a). Assessing soil seed bank persistence in flood-meadows: the search for reliable traits. *J. Veg. Sci.* 15, 93–100. doi: 10.1111/j.1654-1103.2004.tb02241.x

Hölzel, N., and Otte, A. (2004b). Ecological significance of seed germination characteristics in flood-meadow species. *Flora-Morphol. Distribut. Funct. Ecol. Plants* 199, 12–24. doi: 10.1078/0367-2530-00132

Honsova, D., Hejcman, M., Klaudisova, M., Pavlu, V., Kocourkova, D., and Hakl, J. (2007). Species composition of an alluvial meadow after 40 years of applying nitrogen, phospohorus and potassium fertilizer. *Preslia* 79, 245–258.

IPBES (2019). Global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services. Zenodo.

Jakrlová, J. (1999). *Biomass Production of Floodplain Grasslands*. Morava River Floodplain Meadows. DAPHNE, Centre for Applied Ecology, Bratislava, 139–146.

Jefferson, R. G., and Pinches, C. E. (2009). The conservation of floodplain meadows in Great Britain: An overview. *J. Nat. Conserv.* 14, 11–24.

Jõgar, Ü., and Moora, M. (2008). Reintroduction of a rare plant (Gladiolus imbricatus) population to a river floodplain—how important is Meadow Management? *Restor. Ecol.* 16, 382–385. doi: 10.1111/j.1526-100X.2008.00435.x

Joyce, C. B., and Wade, P. M. (1998). *European Wet Grasslands: Biodiversity, Management and Restoration*. Chichester (New York): John Wiley and Sons Ltd.

Kapfer, A. (2010). Mittelalterlich-frühneuzeitliche Beweidung der Wiesen Mitteleuropas. *Naturschut. Landschaftsplanung* 42, 180–187.

Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L., and Hölzel, N. (2010). Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in central and northwestern Europe. *Basic Appl. Ecol.* 11, 285–299. doi: 10.1016/j.baae.2009.12.004

Kirchgeßner, M., and Kellner, R. J. (1982). Schätzung des energetischen Futterwetes von Grün-und Rauhfutter durch die Cellulase-Methode. *Landwirtsch. Forsch.* 34, 276–281.

Klaus, V. H., Hoever, C. J., Fischer, M., Hamer, U., Kleinebecker, T., Mertens, D., et al. (2018). Contribution of the soil seed bank to the restoration of temperate grasslands by mechanical sward disturbance. *Restor. Ecol.* 26, S114–S122. doi: 10.1111/rec.12626

Klaus, V. H., Sintermann, J., Kleinebecker, T., and Hölzel, N. (2011). Sedimentation-induced eutrophication in large river floodplains—an obstacle to restoration? *Biol. Conserv.* 144, 451–458. doi: 10.1016/j.biocon.2010.09.031

Kleinebecker, T., Klaus, V. H., and Hölzel, N. (2011). Reducing sample quantity and maintaining high prediction quality of grassland biomass properties with near infrared reflectance spectroscopy. *J. Near Infrared Spectrosc.* 19, 495–505. doi: 10.1255/jnirs.957

Klinger, Y. P., Eckstein, R. L., Hansen, W., Kleinebecker, T., Otte, A., and Ludewig, K. (2021). Mowing machinery and migratory sheep herds are complementary dispersal vectors for grassland species. *Appl. Veg. Sci.* 24:e12579. doi: 10.1111/avsc.12579

Klinger, Y. P., Eckstein, R. L., Horlemann, D., Otte, A., and Ludewig, K. (2020). Germination of the invasive legume Lupinus polyphyllus depends on cutting date and seed morphology. *Neo Biota* 60, 79–95. doi: 10.3897/neobiota.60.56117

Klotz, S., Kühn, I., and Durka, W. (2002). *BIOLFLOR–eine Datenbank zu biologisch-ökologischen Merkmalen der Gefäßpflanzen in Deutschland. Bundesamt für Naturschutz*. Bonn: Schriftenreihe für Vegetationskunde

Kozak, M., and Piepho, H.-P. (2018). What's normal anyway? Residual plots are more telling than significance tests when checking ANOVA assumptions. *J. Agron. Crop Sci.* 204, 86–98. doi: 10.1111/jac.12220

Kuntze, H., Roeschmann, G., and Schwerdtfeger, G. (1994). *Bodenkunde: 188 Tabellen. 5 neubearb. und erw. 5th Edn.* Stuttgart: Ulmer.

Leyer, I., and Wesche, K. (2008). *Multivariate Statistik in der Ökologie: Eine Einführung*. Berlin and Heidelberg: Springer.

Ludewig, K., Hansen, W., Klinger, Y. P., Eckstein, R. L., and Otte, A. (2021). Seed bank offers potential for active restoration of mountain meadows. *Restor. Ecol.* 29:e13311. doi: 10.1111/rec.13311

Mathar, W., Kleinebecker, T., and Hölzel, N. (2015). Environmental variation as a key process of co-existence in flood-meadows. *J. Veg. Sci.* 26, 480–491. doi: 10.1111/jvs.12254

Metzing, D., Hofbauer, N., Ludwig, G., and Matzke-Hajek, G. (2018). "Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands," in *Band 7: Pflanzen* (Bonn-Bad Godesberg: Bundesamt für Naturschutz).

National Research Council (1999). *Nutrient Requirements of Horses. 5th Edn.* Washington DC: National Academy Press.

Norfolk Wildlife Trust (n.d.). Guidance notes for recording DAFOR. Available at: https://www.norfolkwildlifetrust.org.uk/documents/downloads/cwa/handout-9-using-dafor (Accessed November 19, 2020).

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). Vegan: Community ecology package (2.5–7). Available at: https://CRAN.R-project.org/package=vegan

Poulsen, J. B., Hansen, F., Ovesen, N. B., Larsen, S. E., and Kronvang, B. (2014). Linking floodplain hydraulics and sedimentation patterns along a restored river channel: river Odense, Denmark. *Ecol. Eng.* 66, 120–128. doi: 10.1016/j.ecoleng.2013.05.010

Pywell, R. F., Bullock, J. M., Hopkins, A., Walker, K. J., Sparks, T. H., Burke, M. J. W., et al. (2002). Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *J. Appl. Ecol.* 39, 294–309. doi: 10.1046/j.1365-2664.2002.00718.x

Pywell, R. F., Bullock, J. M., Tallowin, J. B., Walker, K. J., Warman, E. A., and Masters, G. (2006). Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints: enhancing diversity of species-poor grasslands. *J. Appl. Ecol.* 44, 81–94. doi: 10.1111/j.1365-2664.2006.01260.x

Resch, M. C., Schütz, M., Graf, U., Wagenaar, R., van der Putten, W. H., and Risch, A. C. (2019). Does topsoil removal in grassland restoration benefit both soil nematode and plant communities? *J. Appl. Ecol.* 56, 1782–1793. doi: 10.1111/1365-2664.13400

Rodwell, J. S. (1992). "Grasslands and montane communities," in *British Plant Communities. Vol. 3.* (Cambridge: Cambridge University Press).

Roth, F. X., Schwarz, F. J., Stangl, G. I., and Kirchgeßner, M. (2011). *Tierernährung: Leitfaden für Studium, Beratung und Praxis. 13 Edn.* Frankfurt (Main): DLG-Verlag.

Rothero, E., Lake, S., and Gowing, D. (2016). *Floodplain Meadows: Beauty and Utility—A Technical Handbook*. Milton Keynes: Floodplain Meadows Partnership, The Open University.

Schmiede, R., Handke, K., Harnisch, M., Donath, T. W., and Otte, A. (2010). Endbericht: Grundlagen für ein Handlungskonzept zur floristischen und faunistischen Anreicherung artenarmer Auenwiesen. Available at: https://www.dbu.de/OPAC/ab/DBU-Abschlussbericht-AZ-23329.pdf

Schmiede, R., Otte, A., and Donath, T. W. (2012). Enhancing plant biodiversity in species-poor grassland through plant material transfer—the impact of sward disturbance. *Appl. Veg. Sci.* 15, 290–298. doi: 10.1111/j.1654-109X.2011.01168.x

Schumacher, D., and Wolfgang, S. (2016). Naturschutzheu für Milchviehbetriebe? *Land InForm* 6, 36–39.

Sinclair, D. (2016). S-hull: a fast radial sweep-hull routine for Delaunay triangulation. arXiv [Preprint]. Available at: http://arxiv.org/abs/1604.01428

Society for Ecological Restoration (2004). The SER international primer on ecological restoration.

Stöckli, A., Slodowicz, D., Arlettaz, R., and Humbert, J.-Y. (2021). Transfer of invertebrates with hay during restoration operations of extensively managed grasslands in Switzerland. *J. Insect Conserv.* 25, 189–194. doi: 10.1007/s10841-020-00282-8

Tallowin, J. R. B., and Jefferson, R. G. (1999). Hay production from lowland semi-natural grasslands: a review of implications for livestock systems. *Grass Forage Sci.* 54, 99–115. doi: 10.1046/j.1365-2494.1999.00171.x

UN General Assembly (2019). Resolution adopted by the general assembly on 1 march 2019

Valkó, O., Rádai, Z., and Deák, B. (2022). Hay transfer is a nature-based and sustainable solution for restoring grassland biodiversity. *J. Environ. Manag.* 311:114816. doi: 10.1016/j.jenvman.2022.114816 van der Maarel, E. (1979). Transformation of cover-abundance values in phytosociology and its effects on community similarity. *Vegetatio* 39, 97–114. doi: 10.1007/BF00052021

Vécrin, M. P., Grévilliot, F., and Muller, S. (2007). The contribution of persistent soil seed banks and flooding to the restoration of alluvial meadows. *J. Nat. Conserv.* 15, 59–69. doi: 10.1016/j.jnc.2006.10.001

Wagner, M., Hulmes, S., Hulmes, L., Redhead, J. W., Nowakowski, M., and Pywell, R. F. (2021). Green hay transfer for grassland restoration: species capture and establishment. *Restor. Ecol.* 29:e13259. doi: 10.1111/rec.13259

Waldén, E., and Lindborg, R. (2016). Long term positive effect of grassland restoration on plant diversity—success or not? *PLoS One* 11:e0155836. doi: 10.1371/journal.pone.0155836

Wasserstraßen-und Schifffahrtsverwaltung des Bundes (WSV) (2021). Wasserstandsdaten der Rheinpegel Worms, Nierstein-Oppenheim und Mainz, bereitgestellt durch die Bundesanstalt für Gewässerkunde (BfG).

Wesche, K., Krause, B., Culmsee, H., and Leuschner, C. (2012). Fifty years of change in central European grassland vegetation: large losses in species richness and animal-pollinated plants. *Biol. Conserv.* 150, 76–85. doi: 10.1016/j.biocon.2012.02.015

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 26 January 2023
DOI 10.3389/fevo.2023.1107987

OPEN ACCESS

EDITED BY
Péter Török,
University of Debrecen,
Hungary

REVIEWED BY
Francis Isselin,
UMR7324 Cités, Territoires, Environnement et Sociétés (CITERES), France
Triin Reitalu,
University of Tartu,
Estonia

*CORRESPONDENCE
Martina Köhler
✉ martina.koehler@hs-anhalt.de

SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 25 November 2022
ACCEPTED 10 January 2023
PUBLISHED 26 January 2023

CITATION
Köhler M, Schmidt A, Hölzel N, Baasch A and Tischew S (2023) Positive long-term effects of year-round horse grazing in orchid-rich dry calcareous grasslands–Results of a 12-year study.
Front. Ecol. Evol. 11:1107987.
doi: 10.3389/fevo.2023.1107987

Positive long-term effects of year-round horse grazing in orchid-rich dry calcareous grasslands–Results of a 12-year study

Martina Köhler*, Annika Schmidt, Norbert Hölzel, Annett Baasch and Sabine Tischew

Nature Conservation and Landscape Planning, Department of Agriculture, Ecotrophology and Landscape Development, Anhalt University of Applied Sciences, Bernburg, Germany

Introduction: Dry calcareous grasslands are among the most species-rich habitats worldwide but strongly endangered by abandonment causing a severe decline of characteristic species such as orchids. To counteract further degradation, economically sustainable restoration tools such as megaherbivore grazing, that aim to substitute extinct wild grazers, should be considered. However, the long-term effects on target species of dry grasslands, and in particular for orchid populations, is still unclear.

Methods: To contribute to this knowledge gap, we applied vegetation surveys (5m × 5m), as well as large-scale census-based orchid observation and mapping of habitat structures (50m × 50m) in a year-round grazing scheme of a Natura 2000 site in Central Germany over 12 years. The horses and their grazing activity were observed *via* GPS telemetry. We fitted linear mixed models to evaluate whether *Ophrys apifera* density was affected by horse feeding frequency, habitat structure variables (bare soil patches, woody plant cover), grassland type or years.

Results: A The main results were that the target dry calcareous grassland vegetation significantly increased in species numbers and cover. The total abundance of *O. apifera* increased from 1,237 (2013) and 1,893 (2018), to 4,652 (2021) individuals. *Ophrys apifera* density was positively affected by horse feeding frequency as well as grazing-induced enhanced bare soil patches but underlying mechanisms varied between the three classified grassland types.

Discussion: Our results indicate that low-intensity year-round horse grazing as a relatively new restoration tool in dry calcareous grasslands has the potential to enhance floristic biodiversity in general, and particularly *O. apifera* density in the long run. Furthermore, we showed that highvalue xeric grasslands with outstanding orchid abundances can be integrated into the year-round grazing system and that there is no need of fencing off such sections.

KEYWORDS

***Ophrys apifera*, Orchidaceae, plant conservation, management, Konik horses, Natura 2000, remote sensing**

1. Introduction

Dry calcareous grasslands are among the most species-rich habitats of the European cultural landscape (Poschlod and WallisDeVries, 2002; Wilson et al., 2012). They are an essential refugium for low-competitive and often endangered species, such as orchids. Due to their high importance, these grasslands are to be maintained and developed *via* the European Natura 2000 network (Habitats Directive 92/43/EEC). Many dry calcareous grasslands currently show severe management deficits, mainly grass and/or shrub encroachment, due to the widespread cessation of domestic (seasonal) livestock grazing during the last century that has led to a loss of habitat function and biodiversity (Poschlod and WallisDeVries, 2002).

In the last two decades, year-round grazing systems with large herbivores aiming to substitute extinct wild grazers (Vera, 2000; Bunzel-Drüke et al., 2008; Svenning et al., 2016; Pedersen et al., 2020) are increasingly applied to counteract further degradation of nutrient-rich (Gilhaus et al., 2014) and nutrient-poor acidic grasslands or heathlands (Schwabe et al., 2013; Rupprecht et al., 2016; Henning et al., 2017a,b). For these habitats, large herbivores are known to shape the pasture through their specific feeding behavior and utilization (Putfarken et al., 2008; Gilhaus et al., 2014) creating diverse vegetation structures, appropriate biomass removal and improved light conditions at the soil surface through reduced litter layer and woody plant cover (Bokdam and Gleichnam, 2000; Hejcman et al., 2005; Borer et al., 2014). Periodic creation of bare soil patches by feeding and trampling can successfully reactivate the seed bank or provide better recruitment conditions especially for rare species with a high light requirement in the germination and establishment phase (Tischew et al., 2017; Elias et al., 2018), such as orchids (Hutchings, 2010).

EU Natura 2000 legislation has identified calcareous grasslands shaped by human intervention such as grazing and mowing as prime habitat for orchids (Olmeda et al., 2019). At the same time, orchids are believed to be very sensitive to disturbance (e.g., Catorci et al., 2013). Grazing before or during flowering is generally assumed to be (at least at some point) harmful to orchid species due to the mechanical destruction of sensitive leaves and inflorescences (Calaciura and Spinelli, 2008; Catorci et al., 2013). Hence, orchid-rich dry calcareous grasslands are often not integrated into grazing concepts due to expected damage by feeding and trampling through grazing animals, but are mown instead. Especially in the case of year-round grazing, negative grazing effects on orchid species are expected in the winter and spring months (Olmeda et al., 2019), as many species form leaf rosettes during this time. As a consequence, the most commonly applied management of orchid-rich habitats currently is single mowing or grazing within a vegetation season, ideally, after flowering and fruit set (e.g., Jersáková et al., 2015), while more frequent or year-round grazing is mostly considered as harmful and unsuitable. This point of view is often neglecting that grazing could provide open canopy gaps that are important for the regeneration of low-competitive and light demanding orchid species such as *Ophrys apifera*. This addresses the long-debated question in conservation of whether disturbance affects rare species generally in a negative or, in adjusted intensities, in a positive way and which mechanisms operate in this context in the long run (Grubb, 1977). However, only few studies investigated the dynamics of orchid populations under grazing regimes over several years revealing contrasting results. Hutchings (2010) recorded an increase of *Ophrys sphegodes* after the beginning of seasonal sheep grazing in a 32-year study, whereas Catorci et al. (2013) proclaimed spring grazing as threat for orchid species in general in a replicate study after 30 years. So far, Köhler et al. (2016) was the only study finding positive effects on the orchid species *O. apifera* in a year-round grazing system in a calcareous grassland. However, this study covered only 5 years and has raised questions about the long-term effects of year-round megaherbivore grazing on target species of calcareous grasslands.

This current study represents the rare case of a validation of findings from the early years of a large-scale real-life field experiment. In addition to the much longer observation period of 12 years, we observed in details the effects of habitat structures (litter, bare soil, herb layer and woody plant cover) and horse feeding frequency on the development of *O. apifera*. Factors crucial for target orchid development were determined in three different calcareous grassland types (xeric, dry, and dry-mesic). We addressed the following questions:

1. How does the small-scale dry calcareous grassland vegetation develop in terms of the number and abundance over the 12-year grazing period?
2. How does the population of the most important orchid target species *O. apifera* develop in the different calcareous grassland types over the grazing period and how is the density of the orchid affected by horse feeding frequency or habitat structures?

2. Materials and methods

2.1. Study site and study species

The study area comprises the 90 ha horse pasture in the nature reserve and Natura 2000 site “Tote Täler südwestlich Freyburg” in Central Germany (51°13′*N*; 11°46′*E*), *ca.* 250 m above sea level (see also Köhler et al., 2016). The climate is slightly continental with mild winters and warm, relatively dry summers (mean annual temperature: 8.3°C; mean annual precipitation: 565 mm). Shallow loess Luvisols on middle Muschelkalk prevail in the hilly region (Reichhoff et al., 2001).

The study area, surrounded by deciduous forests and arable land, is characterized by a plain, semi-open landscape formed by large-scale dry calcareous grasslands (Festuco-Brometea), patches of shrubs and trees as well as scattered old orchards and several historical small-sized sparsely vegetated quarries (Köhler et al., 2016). From 1950 to 1992 the area was used as a Soviet military training ground. After that period sheep herding was introduced to maintain the calcareous grasslands, but habitat quality decreased because of irregular grazing management with too low stocking rates and biomass removal, leading to highly grass encroached stands dominated by tall grasses such as *Arrhenatherum elatius*, *Bromus erectus* and *Brachypodium pinnatum* (Köhler et al., 2016). As a consequence, the floristic species composition typical of the habitat type 6210* orchid-rich semi-natural dry grasslands on calcareous substrates decreased in extent and quality. Further habitat information is given in Köhler et al. (2016).

In the study area, *O. apifera* Huds., the Bee Orchid has one of the largest regional populations. The critically endangered species of submediterranean distribution occurs on basophilic dry grasslands and light-rich forests (Jäger, 2017) but also is a major colonizer of former quarries and road verges (Gardiner and Vaughan, 2009). Currently, the species is expanding its range to the north, probably due to climate warming (in Saxony-Anhalt since about 1990: Meysel, 2011; in Poland since 2010: Osiadacz and Kręciała, 2014). *Ophrys apifera* is a long-lived perennial wintergreen geophyte and terrestrial orchid that is self-pollinating (Darwin, 1877) at its northern range of distribution (study area), but otherwise bee pollinated by the solitary bee genus *Eucera* (Kullenberg and Bergström, 2008). Plants produce one inflorescence with heights of 20–40 cm and 1–12 blossoms in May–June, fruit set begins in June–July (Ziegenspeck, 1936; Jäger, 2017). The plants emerge from tubers in early autumn and leaves continue to grow slowly during winter making the species exposed to grazing for a rather long period.

Leaves die back during flowering. Reproduction occurs by seeds and vegetatively (Lang, 1980).

2.2. Grazing management

In 2009, a year-round grazing system was introduced to manage the *ca.* 90 ha calcareous grassland (Köhler et al., 2016). The Konik Polski horses formed a herd with on average 18 adult horses (one stallion, 17 mares) and five foals. This results in a stocking rate of 0.3 livestock units (LU)/ha/a (assuming that one adult horse is counted as 1 LU and one foal 0.5 LU). In the first years of grazing, higher stocking rates up to 0.4 LU/ha/a helped repressing the encroachment with tall grasses and could be lowered with the improvement of the site. Additionally, single years of higher biomass production due to higher precipitation required slightly higher stocking rates. The robust breed was able to cope with near-natural conditions such as a very low level of additional winter feeding or rare veterinary intervention (further management information see Köhler et al., 2016).

2.3. Data collection and species classification

2.3.1. Small-scale observations of dry calcareous grassland vegetation

To study the vascular plant species composition of the target vegetation, we randomly established eight permanent 5 m × 5 m vegetation survey plots on the strongly grass encroached parts of the pasture in 2009. We conducted vegetation surveys in June/July annually from 2009 to 2015, and in 2018, and 2020. The calculation of conservation value for vascular plant species is based on the red-lists for Germany (Metzing et al., 2018) and Saxony-Anhalt (Frank et al., 2020). Nomenclature of vascular plants follows Jäger (2017).

We classified vascular plant species into the following ecological groups: target forbs, target grasses (both categories were selected according to the characteristic species of calcareous grasslands following the Habitats Directive), other dry grassland species (including rare ecotone species), mesic grassland species, ruderals and woody plants (Jäger, 2017; species list see Supplementary Table SA1). Each species only belongs to one group.

2.3.2. Large-scale observations of *Ophrys apifera*, habitat structures and horse feeding frequency

Ophrys apifera and habitat structures were observed on grid cells. To stratify habitat structure in orchid census observation grid cells, three vegetation types were classified according to the habitat types of the EU Habitats Directive. Differences in vegetation cover and bare soil patches served as proxies for soil depth and grassland productivity. Xeric calcareous grasslands (xeric) on shallow soils harbored characteristic species of basophilic grasslands of the Alysso-Sedion albi, code 6110* and a range of xeric grassland species such as *Galatella linosyris*, *Teucrium botrys* or *T. chamaedrys* with a total area of 11 ha and 13.6% of the pasture, respectively (red sites in Figure 1). Dry calcareous grasslands (dry) covered 64 ha (76.5%) of the pasture and were dominated by *B. pinnatum*, *Festuca rupicola* or *Scabiosa ochroleuca* (Semi-natural dry grasslands and scrubland facies on calcareous substrates Festuco-Brometalia, code 6210*, green sites in Figure 1). Dry-mesic calcareous grasslands (dry-mesic) on slightly deeper and more productive soils were characterized by dry as well as mesic grassland species such as *A. elatius*, *F. rupicola* or *Origanum vulgare*. In the study area, floristic species composition contains not only submediterranean but also a range of continental species. That vegetation type was found on 8 ha (9.9%) of the pasture (blue sites in Figure 1). The study area was divided into 449 50 m × 50 m grid cells using ArcGIS, of which 121 grid cells were randomly selected proportionally to the size of the three calcareous grassland types (number of grid cells on xeric: 17, dry: 96, dry-mesic: 8). Grid cells with larger patches of high woody vegetation and forests (white sites in Figure 1) were excluded from the selection procedures as not appropriate for *O. apifera*. The selected grid cells covered 32% (27.4 ha) of the *ca.* 90 ha pasture.

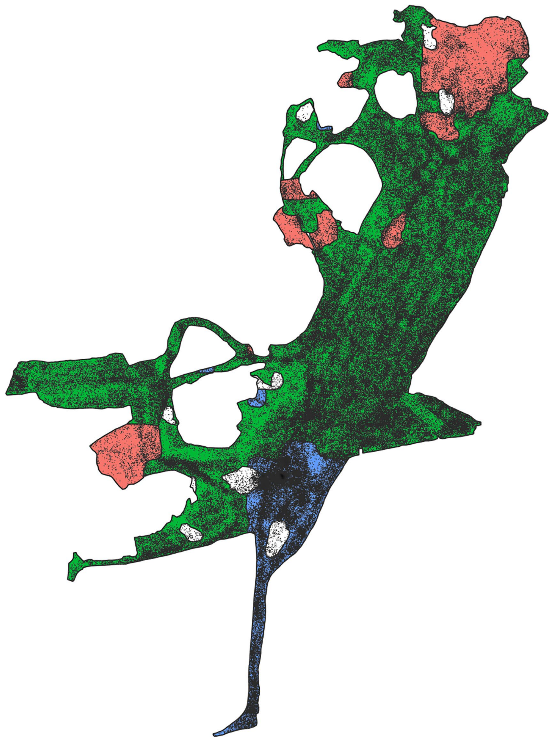


FIGURE 1
Heat map of horse feeding frequency from April–March from 2011 to 2021 (black points correspond to GPS positions). Cover of calcareous grassland types are marked red (xeric), green (dry) and blue (dry-mesic). White sites refer to dense patches of high woody vegetation and forests without remarkable grassland vegetation.

In April of 2013, 2018 and 2021, the number of *O. apifera* individuals at leaf rosette stage was counted in every grid cell in March. Since a few grid cells did not reach full plot size due to pasture fence boundaries, *O. apifera* density was calculated for each grid cell to equal the slightly different grid cell sizes. In 2011, 2012, 2014, 2018, and 2020, habitat structure data (percent cover of litter layer, bare soil patches, herb layer and woody plants) was collected on the grid cells *via* field surveys. Starting in January 2010, the position and activity of the free-ranging horses on the pasture were recorded using remote sensing *via* a GPS PLUS-4 collar (Vectronic Aerospace GmbH Berlin). As the horses showed gregorial behavior, one collar on a female horse recorded spatio-temporal information in 10-min intervals in WGS 84 and UTC time as well as angle and acceleration differences in 2-min intervals *via* a two-axis accelerometer. Inaccuracy of GPS signals due to atmospheric conditions or satellite errors may have resulted in some imprecision of our data. In order to interpret the collar activity data, we collected direct observational behavioral data in the field that was matched to the collar data *via* consistent timestamps (every minute for 881 h on 52 days from 2010 to 2012). During our direct behavioral observations, we did not detect abnormal behavior of the telemetered horse. A discriminant analysis was applied to classify the main behavioral groups feeding, resting and walking of the recorded telemetry data according to the direct observations in the field. As feeding, which always includes walking, was assumed to have the highest impact on the growth cycle of the target orchid species, we selected the horses´ feeding frequency to explain orchid development (Figure 1). The amount of horse feeding frequency data was calculated in each grid cell for one m^2 in ArcGIS (amount of GPS positions classified into feeding per grid cell/grid cell area), as the grid cells cutting the pasture margins showed slightly reduced sizes.

2.4. Statistical analysis

We used non-parametric Friedman tests to test for differences of total species numbers, species numbers and covers within the ecological groups of the small-scale observations of dry calcareous grassland vegetation through time.

Linear mixed models (LMM) were fitted to evaluate whether the density of *O. apifera* was affected by horse feeding frequency, habitat structure variables (bare soil patches, woody plant cover), grassland type or years (Table 1), using lme4 1.1–21 (Bates et al., 2015) and MuMIn 1.43.17 for multi-model selection and averaging (Bartoń, 2020). The cover of the herb as well as litter layer had to be excluded from the modeling process to avoid strong inter-correlations among the predictors ($|r| > 0.6$, Pearson's correlation analysis, $r_{\text{bare soil} \sim \text{herb layer}} = -0.735$, $r_{\text{bare soil} \sim \text{litter layer}} = -0.683$). Moreover, we included all two-way interactions. To achieve normally distributed residuals and avoid heteroscedasticity, we inverse-transformed the density data prior to statistical analysis. Assumptions were checked graphically as recommended by Smith et al. (2009). Possible dependence in the data due to repeated measurements in the same permanent grid cells was controlled by incorporating grid number as random variable in the models. Multi-model selection was based on Akaike information criterion (AIC). For model averaging, we selected all models with ΔAIC < 4 compared to the best model according to AIC.

We implemented all statistical analyzes in R version 4.0.2 (R Core Team, 2020), all telemetry data analyzes in SPSS version 20.0 (IBM Corp. Released, 2011; ArcGIS® 10.1 Software by Esri, 2012).

3. Results

3.1. Development of dry calcareous vegetation under year-round grazing (small scale)

In total, we observed 134 vascular plant species within the eight 5 m × 5 m vegetation survey plots during the entire period of observation, among them 13 endangered red list species (Supplementary Table SA1). Total mean species number significantly increased from 30.3 ± 3.5 to 38.5 ± 2.9 (mean ± SE; df = 8, Qui-square = 41.717, $p < 0.001$, Friedman test).

Different ecological species groups showed varying effects in response to horse grazing. In total, 35 target for species were recorded on all plots with significantly increasing species numbers from 9.9 ± 1.5 to 14.5 ± 1.5 (mean ± SE; Figure 2). In addition, mean number of other dry grassland species and ruderals significantly increased, whereas mesic grassland species significantly decreased (Figure 2). Mean number of target grasses and woody plants remained constant.

From 2009 to 2020, cumulative cover of target forbs, e.g., *Asperula cynanchica*, *Hippocrepis comosa*, *Potentilla neumanniana*, *S. ochroleuca* or *Thesium linophyllon*, as well as other dry grassland species and ruderals significantly increased by 18.1, 4.4 and 2.6%, respectively (Figure 3). On the contrary, cover of ecological species groups indicating abandonment of dry calcareous grasslands such as target grasses and mesic grassland species significantly decreased by 24.2 and 5.6%, respectively (Figure 3). Grass encroachment was mainly built of the target grass species *B. pinnatum* and *B. erectus*. Mean cover of woody plants remained constant on the small-scale plots (Figure 3).

3.2. Development of the target orchid species *Ophrys apifera* under year-round grazing (large scale)

3.2.1. Habitat structures and horse feeding frequency

For large-scale grid cell observation, the study site was stratified in three different grassland types covering xeric, dry and dry-mesic

TABLE 1 Results of the linear mixed-effect model for effects of horse feeding frequency, woody plant cover, bare soil patches, year (three-level factor) and grassland type (three level factor) on density of the target orchid species *Ophrys apifera*.

Density *Ophrys apifera*	Estimate	SE	*P*
Intercept	−41.727	11.673	<0.001
Mean woody plant cover	−6.374	2.465	0.010
Mean horse feeding frequency	42.843	10.879	<0.001
Mean cover bare soil patches	8.144	2.806	0.004
Dry grassland type	−41.735	12.065	0.001
Dry-mesic grassland type	−45.078	17.317	0.009
Year 2021	29.042	6.605	<0.001
Mean horse feeding frequency: dry grassland type	−50.294	11.130	<0.001
Mean horse feeding frequency: dry-mesic grassland type	−44.216	11.556	<0.001
Dry-mesic grassland type: year 2021	−27.434	11.115	0.014

Estimates of model-averaged coefficients are shrinkage estimates including all selected models from model averaging (full model average). Non-significant factors are not displayed but can be found in Supplementary Table SA2. Intercept: Year 2013, xeric grassland type.

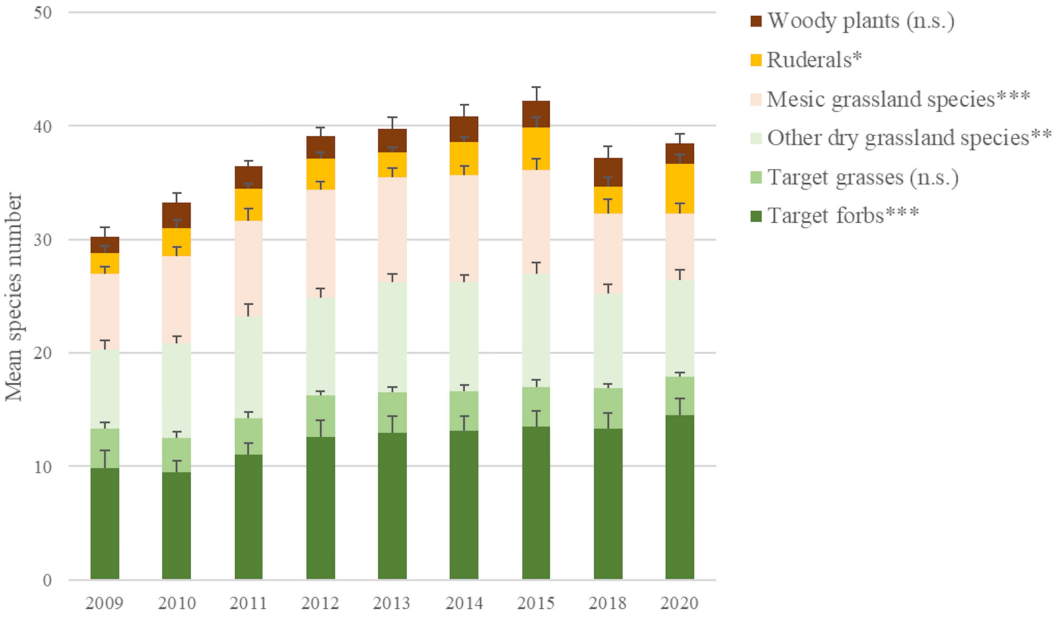


FIGURE 2
Mean vascular plant species number on grazed vegetation survey plots (5m×5m, *n*=8). Significant changes in ecological groups over 9 observational years (2009 to 2015, 2018, 2020) are marked with asterisks (n.s. not significant, **p*<0.05, ***p*<0.01, ****p*<0.001; Friedman tests: Target forbs: df=8; Qui-square=37.479; *p*<0.001; target grasses: df=8; Qui-s. = 5.539; *p*=0.699; other dry grassland species: df=8; Qui-s. = 25.071; *p*=0.002; mesic grassland species: df=8; Qui-s. = 31.212; *p*<0.001; ruderals: df=8; Qui-s. = 15.550; *p*=0.049; woody plants: df=8; Qui-s. = 11.250; *p*=0.188). Error bars indicate standard error.

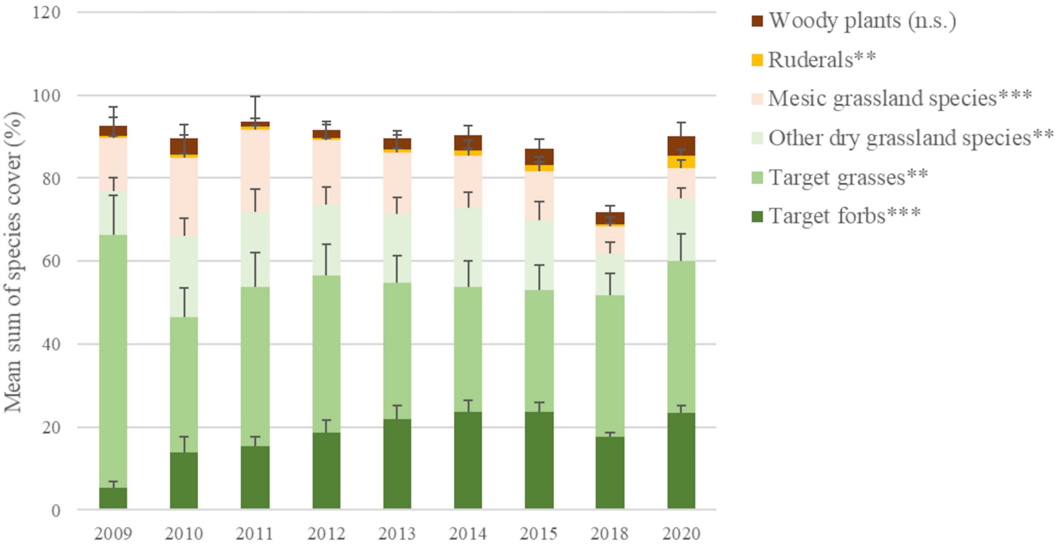


FIGURE 3
Mean cumulative cover of vascular plant species in grazed vegetation surveys (5m×5m, *n*=8). Significant changes in ecological groups over 9 observational years (2009 to 2015, 2018, 2020) are marked with asterisks (n.s. not significant, **p*<0.05, ***p*<0.01, ****p*<0.001; Friedman tests: Target forbs: df=8; Qui-s. = 36.800; *p*<0.001; target grasses: df=8; Qui-s. = 23.933; *p*=0.002; other dry grassland species: df=8; Qui-s. = 25.700; *p*=0.001; mesic grassland species: df=8; Qui-s. = 30.407; *p*<0.001; ruderals: df=8; Qui-s. =21.201; *p*=0.007; woody plants: df=8; Qui-s. = 12.865; *p*=0.117). Error bars indicate standard error.

calcareous grasslands. Litter layer decreased in all calcareous grassland types but most in the dry grassland grid cells. Highest reduction occurred in the first years of grazing, before reaching a stable level of 15–20% in 2018 and 2020 (Figure 4). Area of bare soil patches showed highest values of about 55% in xeric grasslands from the beginning of the survey, whereas in both, dry and dry-mesic grasslands, values were half these maximum values but increased over time (Figure 4). In general, litter layer decreased while herb layer increased. Highest increase in herb layer occurred in the dry grasslands, while dry-mesic grasslands remained constant at *ca.* 60–70% cover (Figure 4). Herb layer in xeric grasslands only slightly increased with significant values since 2018. According to the large-scale observation, woody plant cover was slightly higher in xeric grassland grid cells than in dry or dry-mesic grasslands where horse feeding frequency was highest (Figures 4, 5). In

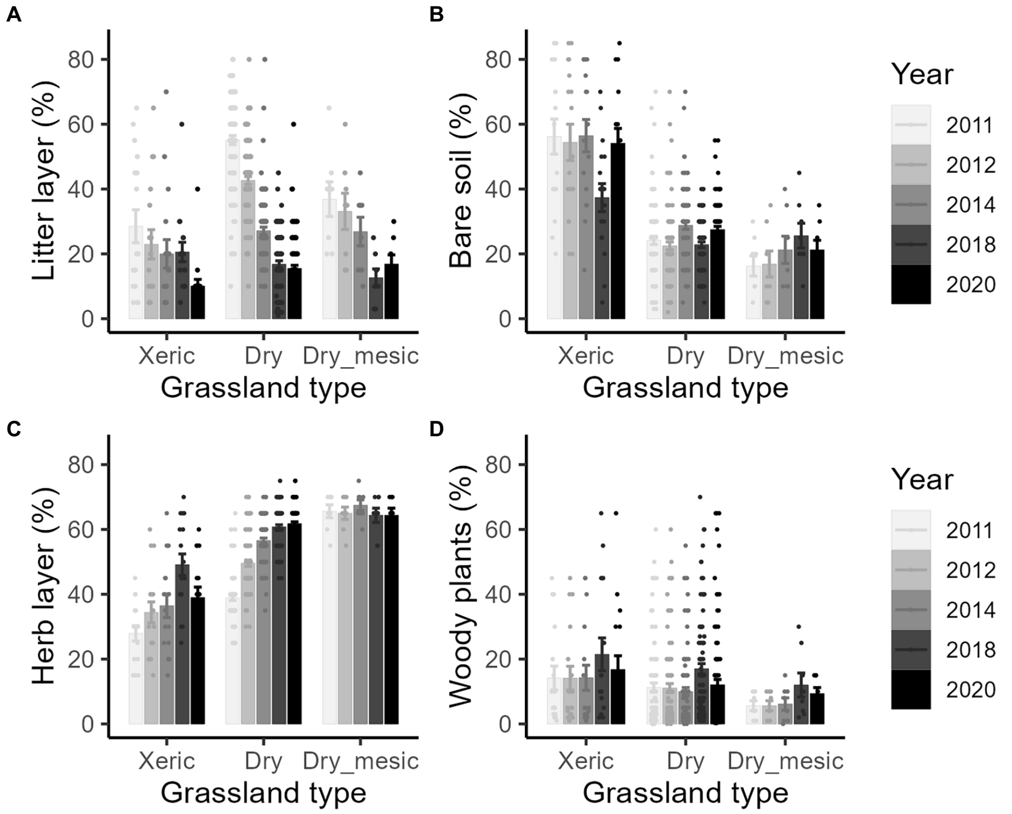


FIGURE 4
Mean values (± SE) of percent cover of habitat structures litter layer **(A)**, bare soil **(B)**, herb layer **(C)** and woody plants **(D)** on the grid cells (50m×50m) in the year-round horse pasture in the different calcareous grassland types (*n*=121; xeric *n*=17, dry *n*=96, dry-mesic *n*=8) in the years 2011, 2012, 2014, 2018 and 2020. Points correspond to the respective data sets.

all vegetation types woody plant cover remained constant at about 15% until 2017 and only slightly increased in 2018.

Horse feeding frequency in xeric grassland grid cells showed lowest means and did not markedly change over the entire grazing period (Figures 1, 5). In grid cells harboring dry calcareous grasslands horse feeding frequency was twice as high compared to xeric grasslands, while highest horse feeding frequency was recorded in dry-mesic grassland grid cells with varying mean values over the period of observation (Figures 1, 5). In 2020, lower values correspond with the partial absence of horses (due to low fodder availability related to the extreme drought).

3.2.2. Development of *Ophrys apifera* and effects of habitat structures and horse feeding frequency

The total number of recorded individuals of the target orchid species *O. apifera* in the 121 grid cells continuously and significantly increased from 1,237 in 2013 and 1,893 in 2018, to 4,652 in 2021. Hence, the total number of individuals clearly increased, and was estimated to be at least 10,000 individuals in the entire pasture in 2021.

Ophrys apifera density significantly varied in the different calcareous grassland types showing highest values in xeric, medium in dry and lowest in dry-mesic grassland grid cells (Table 1; Figure 6). Within the observation period, density doubled in xeric (almost) and dry grassland (significantly) grid cells from 2018 to 2021 (Figure 6). Density in dry-mesic grasslands remained stable from 2013 to 2021. The total number of *O. apifera* individuals was highest in dry, and twice as high as in xeric grassland grid cells.

Horse feeding frequency affected the density of *O. apifera* positively but showed varying effects between grassland types (Table 1; Figures 7, 8; Supplementary Table SA2). In xeric grasslands, grid cells with higher horse feeding frequency also had a higher *O. apifera* density compared to xeric grassland grid cells of low horse feeding frequency. In contrast, in dry-mesic grasslands which generally exhibited the highest horse feeding frequency (compared to the xeric and dry grassland type), the grid cells with a higher *O. apifera* density tended to be frequented less often. The increase of *O. apifera* on dry grasslands covered a relatively broad range of horse feeding frequency and contributed most to the overall increase of individuals (Figure 7). A high cover of bare soil patches was predicted to have the second largest positive effect. In contrast, grid cells with a higher woody plant cover were predicted to show a lower density of *O. apifera*. High horse feeding frequency was negatively correlated with woody plant cover ($r_{\text{woody plant cover} \sim \text{horse feeding frequency}} = -0.382$, $p = <0.001$, Pearson's correlation coefficient).

4. Discussion

4.1. Year-round grazing effects on small-scale dry calcareous vegetation

We investigated the number and cover of characteristic dry calcareous grassland species over a study period of 12 years in a year-round horse grazing regime. We found a constant increase particularly of target forbs which only decreased in cover due to the severe drought

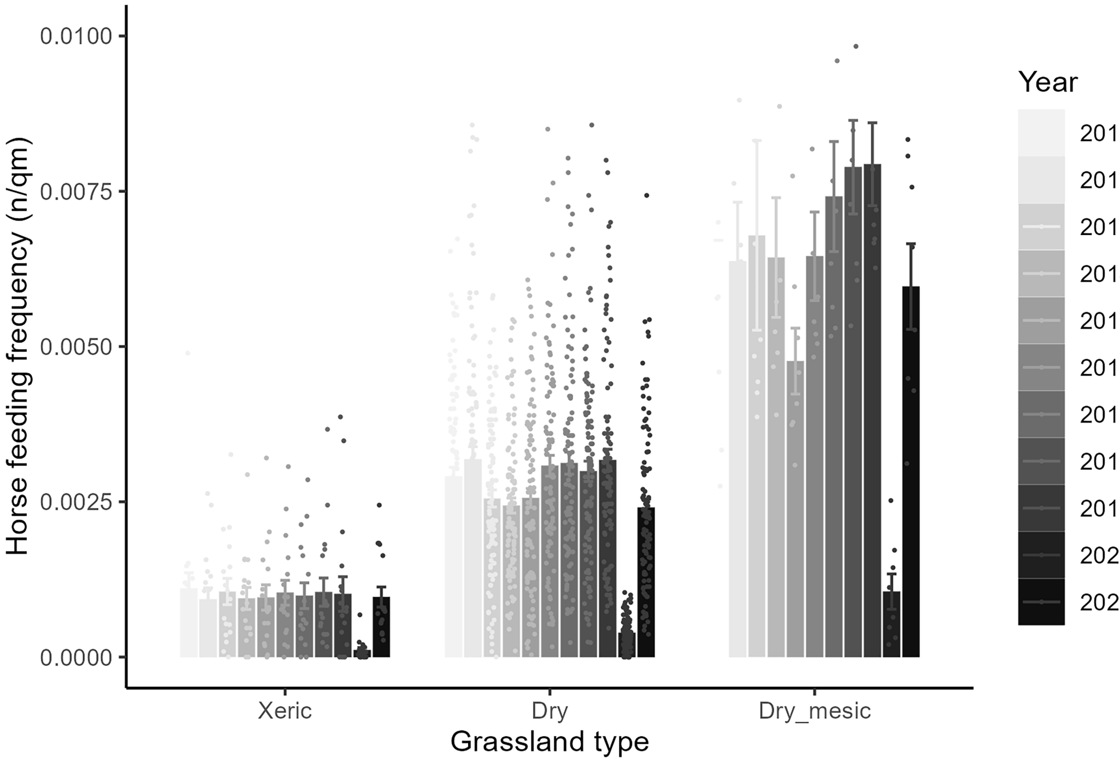


FIGURE 5
Mean values (± SE) of horse feeding frequency per qm on the grid cells in the different calcareous grassland types (*n*=121; xeric *n*=17, dry *n*=96, dry-mesic *n*=8) from April–March from 2011–2021. Lower values in 2020 referred to partial absence of horses (due to low fodder availability related to the extreme drought). Points correspond to the respective single data sets.

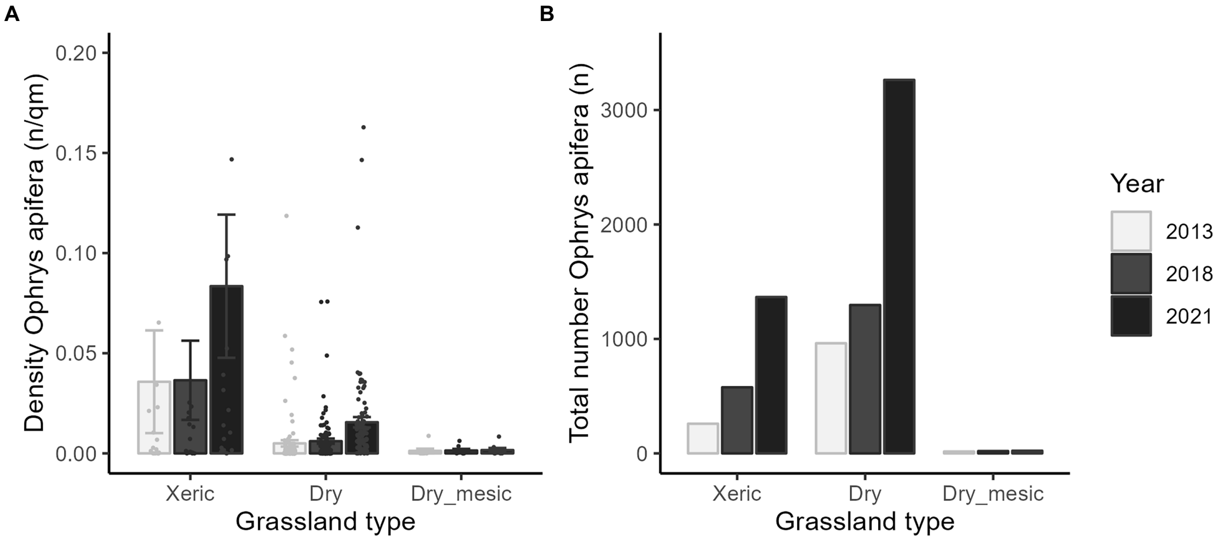


FIGURE 6
Mean values (± SE) of the density **(A)** and total number **(B)** of *Ophrys apifera* on the grid cells in the different calcareous grassland types (*n*=121; xeric *n*=17, dry *n*=96, dry-mesic *n*=8) in the years 2013, 2018 and 2021. Values 0.546, 0.440 and 0.347 **(A)** and 407 **(B)** are not displayed. Points correspond to the respective data sets.

in Central Germany in 2018, whereas the cover of target grasses significantly decreased. Low competitive dry grassland species depending on bare soil patches benefited from grazing and increased. This finding emphasizes that the typical floristic species composition can be maintained and even improved by year-round grazing not only over short periods (Köhler et al., 2016), but also in the long run. Several studies have reported a positive impact of year-round equid grazing on the abundance of target species of dry sandy grasslands (Süss and

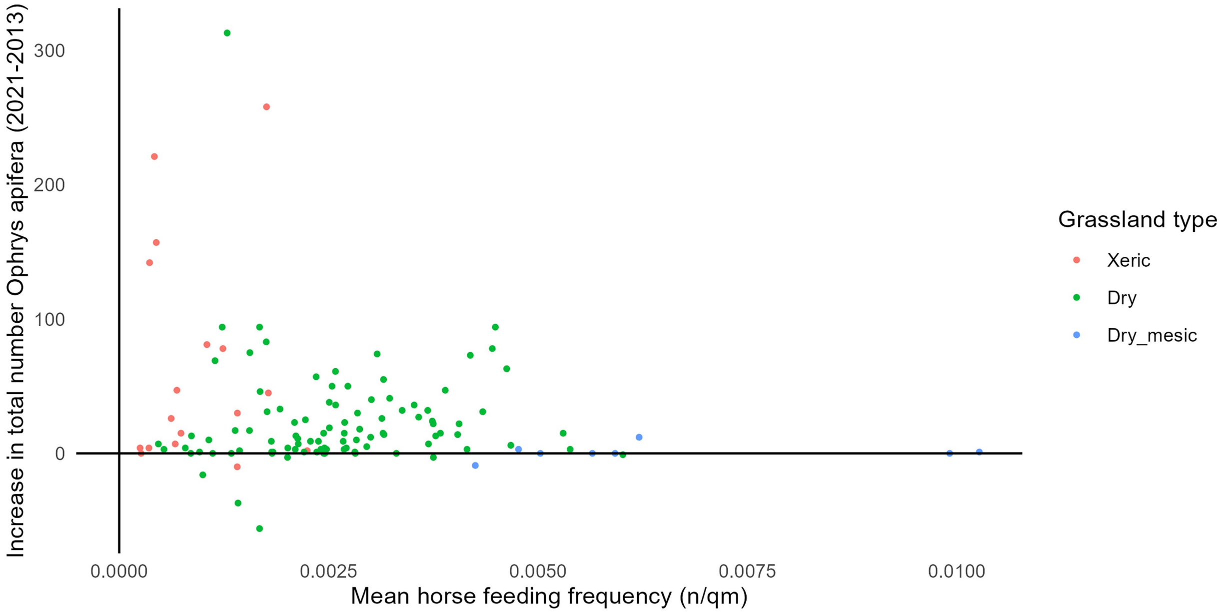


FIGURE 7
Increase in total number of *Ophrys apifera* from 2013 to 2021 and mean horse feeding frequency from April–March from 2011–2021 on the grid cells in the different calcareous grassland types (*n*=121; xeric *n*=17, dry *n*=96, dry-mesic *n*=8).

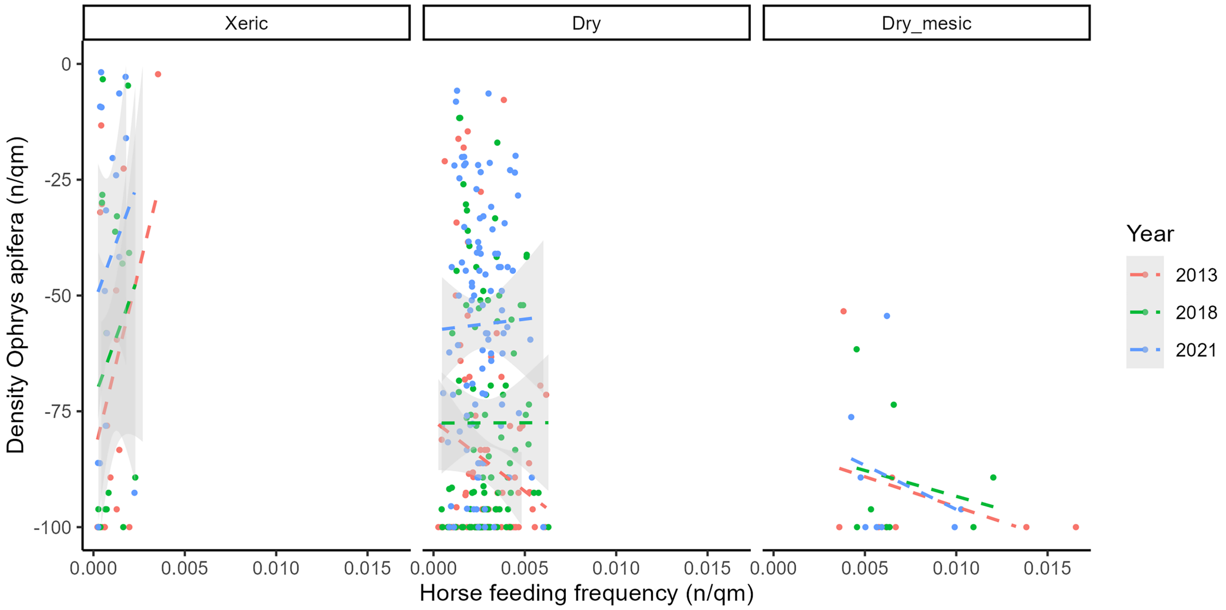


FIGURE 8
Effect of horse feeding frequency on inverse-transformed density of *Ophrys apifera* in the different calcareous grassland types in 2013, 2018 and 2021 (*n*=121; xeric *n*=17, dry *n*=96, dry-mesic *n*=8). Shaded area represents 95% confidence intervals.

Schwabe, 2007; Schwabe et al., 2013; Henning et al., 2017a) assuming that a sufficient, adapted stocking rate is guaranteed as undergrazing can lead to ruderalization or tall species dominance (Sýkora et al., 2009; Moinardeau et al., 2021). Concerning faunistic diversity, forb enhancement of grassland is usually beneficial to insects as forbs provide both nesting and foraging sites (Bonari et al., 2017), and in the consequence beneficial to higher trophic animal species like birds (Lovász et al., 2021; Schmidt et al., 2022).

Compared to other grazing animal species such as cattle, sheep or goats, equids can compensate low-quality forage through increased, nearly permanent feeding (Duncan et al., 1990; own observation data). As equids show a preference for grass species, grasses are disproportionately more frequently used compared to forbs. Grass species, almost indigestible for many herbivores due to silicate inclusion in the cells and lignifying fibers, can be digested by equids because of their digestive system geared to fiber utilization and high-crowned teeth protecting from abrasion (coevolution of grasses and grazers beginning in the Tertiary; Stebbins, 1981; Bunzel-Drüke et al., 2008). These characteristics make horses well suited to reduce grass encroachment in dry grasslands (Süss and Schwabe, 2007; Schwabe et al., 2013).

We found that ruderals significantly increased only in the long-term observation period, probably due to enhanced disturbance and more bare soil patches created by the continuous reduction of herb and litter layers through grazing in the long run. The observed short-lived, small-growing, thermophilic ruderal species such as *Arenaria serpyllifolia*, *Cerastium pumilum* or *Draba verna* are seen as intrinsic part of pastures that enhance their biodiversity (Elias et al., 2018; Köhler et al., 2020) as well as nectar and pollen supply for insects, but do not affect the species assemblage of dry grasslands due to the low increase of cover. Extended nitrophilous ruderal patches that are often discussed as problematic in grazed areas, have not been observed in our study, most likely due to the generally low nutrient levels. Generally, the different types of zoochory leading to species dispersal inside the pasture are seen as the main driver of long-time establishment success of target species (Kiss et al., 2021), emphasizing the benefits of grazing compared to mowing.

4.2. Large-scale effects of year-round grazing on *Ophrys apifera*

We evaluated the long-term effects of year-round horse grazing on the target orchid species *O. apifera* in a dry calcareous grassland using data on habitat structures and horse feeding frequency. We found a continuous significant increase of the counted *O. apifera* individuals on most observed grid cells from 2013 to 2021. This important finding means that low-intensity horse grazing is generally suitable for orchid species maintenance and promotion on large-scale, plain dry calcareous grasslands. The orchid species' ability to cope with permanent grazing has already been demonstrated by Köhler et al. (2016) for a 5-year grazing period. In this study, we confirm these results for a 12-year period and clarified relevant mechanisms in the different calcareous grassland types. Across all three calcareous grassland types, horse feeding frequency had an overall positive effect on the density of *O. apifera* individuals. However, *O. apifera* abundance varied inside the pasture due to differently appropriate site conditions. Particularly, the increase of bare soil patches and the reduction of litter layers induced by feeding and trampling led to lower competition through the surrounding vegetation and to enhanced light availability on the ground (Borer et al., 2014) creating niches for orchid recruitment and seedling establishment (Shefferson et al., 2020). In addition, biomass and particularly grass cover reduction also diminished vegetation competition.

Sites characterized by shallow soils, a high share of bare soil patches and low vegetation cover that harbor xeric calcareous grassland species showed highest initial *O. apifera* abundance at the beginning of the study. Here, lowest horse feeding frequencies were recorded over the observation period, as grazers probably avoided grassland patches with both low fodder availability and quality (Gilhaus et al., 2014). However, under this condition of generally very low grazing intensity, the higher the feeding frequency, the more positive it affected *O. apifera* density due to the better reduction of litter layer and the dynamic creation of bare soil patches. Hence, these high-value xeric grasslands can be integrated into the grazing system contrary to often recommended fencing off (Calaciura and Spinelli, 2008; Catorci et al., 2013), at least in large-scaled pastures (> 10, better > 50 ha; Bunzel-Drüke et al., 2008) where sites of higher productivity are preferentially grazed.

Sites, that were initially less suited for orchid occurrence due to heavy grass encroachment and thick litter layers, were characterized by higher vegetation cover of dry calcareous grasslands. These sites were significantly more frequented by the horses due to higher fodder availability and quality. On this grassland type, that had by far the largest area share (80.9%), *O. apifera* density was significantly lower at the beginning of grazing compared to xeric grasslands. But, surprisingly, it also increased significantly and contributed most to the increase in numbers of individuals due to enhanced habitat quality. The benefits of grazing for the development of *O. apifera* in this grassland type were most effective at a medium horse feeding frequency, since grazing frequency did not show an explicit effect on the individual density. On grid cells with a higher grazing frequency, positive grazing effects seemed to be able to outbalance negative effects such as trampling and feeding on *O. apifera*.

Grid cells of the dry-mesic calcareous grassland type were *per-se* sub-optimal sites for orchid occurrence as characterized by deeper soils, supporting more mesic, higher-competitive species. On these (rather small) sites of the pasture, horse feeding frequency showed significantly highest values probably due to higher protein forage compared to dry and xeric grasslands (Gilhaus et al., 2014). But even on these sites, *O. apifera* individual density was stabilized during the horse grazing experiment. Here, grid cells with lower or medium horse feeding frequency maintained *O. apifera* populations, while at higher horse feeding frequency a slightly negative effect was observed (Figure 8).

In contrast to other studies reporting direct negative grazing effects on orchids by feeding and trampling (Calaciura and Spinelli, 2008; Catorci et al., 2013; Cleavitt et al., 2016), we found positive effects of year-round grazing. Horses that intentionally graze orchid inflorescences or leaves were not observed in our study (field observation). *Vice-versa*, horse grazing was shown to maintain or create habitat structures that supported the strong population increase of the target orchid species. A crucial factor for this development was the increase of bare soil patches mainly induced by grazing and trampling. The establishment of many orchid species is not limited by seed but microsite availability (Shefferson et al., 2020). Hutchings (2010) reported site disturbance as a key driver of species recruitment *of O. sphegodes* and Gardiner and Vaughan (2009) outlined that site disturbance at road verges enhanced *O. apifera* abundance. The regular and dynamic creation of bare soil patches might be the main advantage of large herbivore grazing in comparison to mowing or small-scale rudimentary grazing. Intermediate disturbance often initiates positive processes such as species establishment and should not only be discussed as generally problematic in conservation and restoration ecology (Grubb, 1977).

In contrast to other reports of the failure of year-round horse grazing to counteract woody plant encroachment (e.g., Cosyns et al., 2001), our study revealed that woody plant cover only slightly increased under grazing impact in the long term, indicating the potential of horses to prevent significant increase of woody plant cover in low precipitation habitats with relatively low initial woody plant cover. In addition, only a few potentially invasive, stoloniferous woody species were present on the pasture (Köhler et al., 2016) and additional mineral supply was limited enhancing the intensity of browsing (especially bark peeling) to meet the mineral requirements particularly in the winter months (Putfarken et al., 2008). Moreover, only marginal areas needed additional restoration treatment such as mechanical shrub removal to achieve restoration targets compared to other similar pasture regimes (e.g., Henning et al., 2017a). For the positive development of target bird communities, remaining individual shrubs as well as groups of shrubs and small forest patches should be considered as beneficial (Köhler et al., 2016; Lovász et al., 2021).

Besides grazing, there might be other factors influencing orchid density. *Ophrys apifera* shows a general increase and range extension probably due to climate warming in Saxony-Anhalt and Central Europe since the 1990s (Hutchings, 2010; Meysel, 2011; Osiadacz and Kręciała, 2014). Increases to such an extent have not been observed in other pastures in Saxony-Anhalt but the species often even decreased due to poor management. A not yet published dataset of the regional working group Arbeitskreis Heimische Orchideen Saxony-Anhalt e.V. shows clearly that in an area managed by mowing the number of *O. apifera* individuals remained stable over the same period, but did not increase. Yet, clarifying studies about climate impact on population growth and range expansion of *O. apifera* are missing. Fast population development in the pasture may also derive from relatively short reproduction cycles between germination and first appearance of *O. apifera*. Ziegenspeck (1936) suggested that the species can produce leaves after only 1 or 2 years.

4.3. Conclusion

Our results indicated that year-round horse grazing as a relatively new grassland restoration tool has the potential to enhance floristic biodiversity of dry calcareous grasslands not only in the short, but also in the long term. Particularly, the development of orchid species has to be evaluated differentially, when assessing the restoration success. Since pastures usually consist of heterogeneous vegetation types with varying suitability for orchid establishment, practitioners should not only rely on mean values concerning the entire pasture to regulate management but also on decrease and increase of individuals in individual vegetation types as the potential for local new establishment can strongly vary. In our study site, numbers of *O. apifera* increased most significantly in the prevailing medium dry calcareous grassland type and this type contributed most to the overall increase of individuals. Furthermore, our study clearly showed that even high-value xeric grasslands with very high orchid abundances can be integrated into the year-round grazing system also in the long term, and there is no need to the often recommended fencing off (exclosure) of such particularly valuable areas. Our study also revealed that short-term, especially one-year-only evaluation of restoration schemes can be misleading, particularly at slow-changing indicators such as dry calcareous vegetation types and at slow-growing and longer-lived species or those exhibiting high interannual population fluctuations such as many orchid species. Long-term monitoring schemes should be implemented to meet these requirements and to obtain found results on the effects of restoration and management measures.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because our work was a purely observational study and no ethical approval was needed. The GPS collar that provided our horse feeding frequency data was fitted and regularly controlled on one female horse without using any external devices such as sedation. In order to access the telemetry data, no direct animal contact was needed. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MK and ST developed the research questions and plot selection. MK collected the data and wrote the manuscript. MK and AS analyzed the data. AS, AB, NH, and ST contributed critically to the drafts and gave final approval for publication. All authors contributed to the article and approved the submitted version.

Funding

Funding was provided by the European Agricultural Fund for Rural Development (EAFRD) and the state of Saxony-Anhalt (407.1.7–60128/323009000054, 323010000089, 323012000034, and 630116000006) and by the Graduate Scholarship Program of Saxony-Anhalt. We acknowledge support by the German Research Foundation (Deutsche Forschungsgemeinschaft DFG) -project number 491460386 - and the Open Access Publishing Fund of Anhalt University of Applied Sciences.

Acknowledgments

The authors thank Georg Hiller and Frank Meysel as well as all students who helped with the data collection in the field, particularly horse observations, the Nature Conservation Authority Burgenlandkreis for implementing the financial support of the pasture, the Agrar GmbH Crawinkel for implementing the management, the owner of the site Naturstiftung David – Die Stiftung des BUND Thüringen and further cooperation partners.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1107987/full#supplementary-material

References

ArcGIS$^{®}$ 10.1 Software by Esri. (2012). *ArcGIS$^{®}$*.

Bartoń, K. (2020). MuMIn: Multi-Model Inference. R Package Version 1. 43. 17. Available at: https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bokdam, J., and Gleichnam, J. M. (2000). Effects of grazing by free-ranging cattle on vegetation dynamics in a continental north-west European heathland. *J. Appl. Ecol.* 37, 415–431. doi: 10.1046/j.1365-2664.2000.00507.x

Bonari, G., Fajmon, K., Malenovský, I., Zelený, D., Holuša, J., Jongepierová, I., et al. (2017). Management of semi-natural grasslands benefiting both plant and insect diversity: the importance of heterogeneity and tradition. *Agric. Ecosyst. Environ.* 246, 243–252. doi: 10.1016/j.agee.2017.06.010

Borer, E. T., Seabloom, E. W., Gruner, D. S., Harpole, W. S., Hillebrand, H., Lind, E. M., et al. (2014). Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* 508, 517–520. doi: 10.1038/nature13144

Bunzel-Drüke, M., Böhm, C., Finck, P., Kämmer, G., Luick, R., Reisinger, E., et al., (2008). *Praxisleitfaden für Ganzjahresbeweidung im Naturschutz und Landschaftsentwicklung–Wilde Weiden*. Arbeitsgemeinschaft Biologischer Umweltschutz im Kreis Soest e. V., Bad Sassendorf-Lohne.

Calaciura, B., and Spinelli, O.. (2008). *Management of Natura 2000 Habitats. 6210 Semi-natural Dry Grasslands and Scrubland Facies on Calcareous Substrates (Festuco-Brometalia) (*important orchid sites)*. Brussels: European Commission.

Catorci, A., Cesaretti, S., and Gatti, R. (2013). Effect of long-term abandonment and spring grazing on floristic and functional composition of dry grasslands in a central Apennine farmland. *Pol. J. Ecol.* 61, 505–517.

Cleavitt, N. L., Berry, E. J., Hautaniemi, J., and Fahey, T. J. (2016). Life stages, demographic rates, and leaf damage for the round-leaved orchids, *Platanthera orbiculata* (Pursh.) Lindley and *P. macrophylla* (Goldie) PM Brown in a northern hardwood forest in New Hampshire, USA. *Botany* 95, 61–71. doi: 10.1139/cjb-2016-0164

Cosyns, E., Degezelle, T., Demeulenaere, E., and Hoffmann, M. (2001). Feeding ecology of Konik horses and donkeys in Belgian coastal dunes and its implications for nature management. *Belg. J. Zool.* 131, 111–118.

Darwin, C. (1877). *Various Contrivances by which Orchids are Fertilized by Insects*. London: John Murray.

Duncan, P., Foose, T. J., Gordon, I. J., Gakahu, C. G., and Lloyd, M. (1990). Comparative nutrient extraction from forages by grazing bovids and equids. A test of the nutritional model of equid/bovid competition and coexistence. *Oecologia* 84, 411–418. doi: 10.1007/BF00329768

Elias, D., Hölzel, N., and Tischew, S. (2018). Positive effects of goat pasturing on the threatened spring geophyte *Gagea bohemica* in formerly abandoned dry grasslands. *Flora* 249, 53–59. doi: 10.1016/j.flora.2018.09.008

Frank, D., Brade, P., Elias, D., Glowka, B., Hoch, A., John, H., et al. (2020). Rote Listen Sachsen-Anhalt: Farne und Blütenpflanzen (Pteridophyta et Spermatophyta). *Ber. Landesamtes Umweltschutz Sachsen Anhalt* 1, 151–186.

Gardiner, T., and Vaughan, A. (2009). Scrub clearance and soil disturbance increases bee orchid *Ophrys apifera* frequency in calcareous grassland at Norton heath roadside verge, Essex, England. *Conserv. Evid.* 6, 39–41.

Gilhaus, K., Stelzner, F., and Hölzel, N. (2014). Cattle foraging habits shape vegetation patterns of alluvial year-round grazing systems. *Plant Ecol.* 215, 169–179. doi: 10.1007/s11258-013-0287-6

Grubb, P. J. (1977). The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol. Rev.* 52, 107–145. doi: 10.1111/j.1469-185X.1977.tb01347.x

Hejcman, M., Auf, D., and Gaisler, J. (2005). Year-round cattle grazing as an alternative management of hay meadows in the Giant Mts (Krkonose, Karkonosze), the Czech Republic. *Ekol. Bratisl.* 24, 419–429.

Henning, K., Lorenz, A., von Oheimb, G., Härdtle, W., and Tischew, S. (2017a). Year-round cattle and horse grazing supports the restoration of abandoned, dry sandy grassland and heathland communities by supressing *Calamagrostis epigejos* and enhancing species richness. *J. Nat. Conserv.* 40, 120–130. doi: 10.1016/j.jnc.2017.10.009

Henning, K., von Oheimb, G., Härdtle, W., Fichtner, A., and Tischew, S. (2017b). The reproductive potential and importance of key management aspects for successful *Calluna vulgaris* rejuvenation on abandoned continental heaths. *Ecol. Evol.* 7, 2091–2100. doi: 10.1002/ece3.2816

Hutchings, M. J. (2010). The population biology of the early spider orchid *Ophrys sphegodes* mill. III. Demography over three decades. *J. Ecol.* 98, 867–878. doi: 10.1111/j.1365-2745.2010.01661.x

IBM Corp. Released. (2011). *IBM SPSS Statistics for Windows, Version 20.0*. Armonk, NY: IBM Corp.

Jäger, E. J. (eds). (2017). *Rothmaler - Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband. 21st*, Springer-Verlag, Berlin, Heidelberg.

Jersáková, J., Traxmandlová, I., Ipser, Z., Kropf, M., Pellegrino, G., Schatz, B., et al. (2015). Biological flora of Central Europe: *Dactylorhiza sambucina* (L.) Soó. *Perspect. Plant Ecol. Evol. Syst.* 17, 318–329. doi: 10.1016/j.ppees.2015.04.002

Kiss, R., Deak, B., Tothmeresz, B., Miglecz, T., Toth, K., Török, P., et al. (2021). Establishment gaps in species-poor grasslands: artificial biodiversity hotspots to support the colonization of target species. *Restor. Ecol.* 29:e13135. doi: 10.1111/rec.13135

Köhler, M., Elias, D., Hiller, G., Hölzel, N., and Tischew, S. (2020). Restoration of orchid-rich dry calcareous grasslands by rotational goat pasturing. *Tuexenia* 40, 201–223. doi: 10.14471/2020.40.009

Köhler, M., Hiller, G., and Tischew, S. (2016). Year-round horse grazing supports typical vascular plant species, orchids and rare bird communities in a dry calcareous grassland. *Agric. Ecosyst. Environ.* 234, 48–57. doi: 10.1016/j.agee.2016.03.020

Kullenberg, B., and Bergström, G. (2008). Hymenoptera aculeate males as pollinators of *Ophrys orchids. Zool. Script.* 5, 13–23. doi: 10.1111/j.1463-6409.1976.tb00678.x

Lang, D. (1980). *Orchids of Britain. A Field Guide*. Oxford: Oxford University Press.

Lovász, L., Korner-Nievergelt, F., and Amrhein, V. (2021). Grazer density and songbird counts in a restored conservation area. *PeerJ* 9:e10657. doi: 10.7717/peerj.10657

Metzing, D., Garve, E., Matzke-Hajek, G., Adler, J., Bleeker, W., Breunig, T., et al. (2018). "Rote Liste und Gesamtartenliste der Farn- und Blütenpflanzen (Trachaeophyta) Deutschlands" in *Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands. Band 7: Pflanzen*. eds. D. Metzing, N. Hofbauer, G. Ludwig and G. Matzke-Hajek, vol. *70* (Germany: Münster (Landwirtschaftsverlag), Naturschutz und Biologische Vielfalt), 13–358.

Meysel, F. (2011). "*Ophrys apifera* Huds. Bienenragwurz" in *Arbeitskreis Heimische Orchideen Sachsen-Anhalt E.V. Orchideen in Sachsen-Anhalt: Verbreitung, Ökologie, Variabilität, Gefährdung, Schutz* eds. G. Bräuer, C. Heyde, K. Ludwig, H. John and H. J. Kison (Quedlinburg: Druck GmbH) ISBN 978-3-942975-03-2

Moinardeau, C., Mesleard, F., Ramone, H., and Dutoit, T. (2021). Grazing in temporary paddocks with hardy breed horses (Konik polski) improved species-rich grasslands restoration in artificial embankments of the Rhône river (southern France). *Glob. Ecol. Conserv.* 31:e01874. doi: 10.1016/j.gecco.2021.e01874

Olmeda, C., Šefferová, V., Underwood, E., Millan, L., Gil, T., and Naumann, S.. (2019). *EU Action Plan to Maintain and Restore to Favourable Conservation Status the Habitat Type 6210 Semi-natural Dry Grasslands and Scrubland Facies on Calcareous Substrates (Festuco-Brometalia; *important orchid sites)*. European Commission Technical Report.

Osiadacz, B., and Kręciała, M. (2014). *Ophrys apifera* Huds. (Orchidaceae), a new orchid species to the flora of Poland. *Biodiv. Res. Conserv.* 36:11-16 doi: 10.2478/biorc-2014-0027

Pedersen, P. B. M., Ejrnæs, R., Sandel, B., and Svenning, J. C. (2020). Trophic rewilding advancement in Anthropogenically impacted landscapes (TRAAIL): a framework to link conventional conservation management and rewilding. *Ambio* 49, 231–244. doi: 10.1007/s13280-019-01192-z

Poschlod, P., and WallisDeVries, M. F. (2002). The historical and socioeconomic perspective of calcareous grasslands–lessons from the distant and recent past. *Biol. Conserv.* 104, 361–376. doi: 10.1016/S0006-3207(01)00201-4

Putfarken, D., Dengler, J., Lehmann, S., and Härdtle, W. (2008). Site use of grazing cattle and sheep in a large-scale pasture landscape: a GPS/GIS assessment. *Appl. Anim. Behav. Sci.* 111, 54–67. doi: 10.1016/j.applanim.2007.05.012

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Reichhoff, L., Kugler, H., Refior, K., and Warthemann, G. (2001). *Die Landschaftsgliederung Sachsen-Anhalts. Ein Beitrag zur Fortschreibung des Landschaftsprogrammes des Landes Sachsen-Anhalt*. Germany: Magdeburg/Halle.

Rupprecht, D., Gilhaus, K., and Hölzel, N. (2016). Effects of year-round grazing on the vegetation of nutrient-poor grass-and heathlands–evidence from a large-scale survey. *Agric. Ecosyst. Environ.* 234, 16–22. doi: 10.1016/j.agee.2016.02.015

Schmidt, A., Fartmann, T., Kiehl, K., Kirmer, A., and Tischew, S. (2022). Effects of perennial wildflower strips and landscape structure on birds in intensively farmed agricultural landscapes. *Basic Appl. Ecol.* 58, 15–25. doi: 10.1016/j.baae.2021.10.005

Schwabe, A., Süss, K., and Storm, C. (2013). What are the long-term effects of livestock grazing in steppic sandy grassland with high conservation value? Results from a 12-year field study. *Tuexenia* 33, 189–212.

Shefferson, R. P., Jacquemyn, H., Kull, T., and Hutchings, M. J. (2020). The demography of terrestrial orchids: life history, population dynamics and conservation. *Bot. J. Linn. Soc.* 192, 315–332. doi: 10.1093/botlinnean/boz084

Smith, G. M., Saveliev, A. A., Walker, N., Ieno, E. N., and Zuur, A. F. (2009). *Mixed Effects Models and Extensions in Ecology with R. Statistics for Biology and Health S.L.* Berlin: Springer-Verlag. Available at: http://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=275860

Stebbins, G. L. (1981). Coevolution of grasses and herbivores. *Ann. Mo. Bot. Gard.* 68, 75–86. doi: 10.2307/2398811

Süss, K., and Schwabe, A. (2007). Sheep versus donkey grazing or mixed treatment: results from a 4-year field experiment in Armerio-Festucetum trachyphyllae sand vegetation. *Phytocoenologia* 37, 135–160. doi: 10.1127/0340-269X/2007/0037-0135

Svenning, J. C., Pedersen, P. B. M., Donlan, C. J., Ejrnaes, R., Faurby, S., Galetti, M., et al. (2016). Science for a wilder Anthropocene: synthesis and future directions for trophic rewilding research. *Proc. Natl. Acad. Sci. U. S. A.* 113, 898–906. doi: 10.1073/pnas.1502556112

Sýkora, K. V., Stuiver, H. J., de Ronde, I., and de Nijs, L. J. (2009). Fourteen years of restoration and extensive year round grazing with free foraging horses and cattle and its effect particularly on dry species rich riverine levee grasslands. *Phytocoenologia* 39, 265–286. doi: 10.1127/0340-269X/2009/0039-0265

Tischew, S., Kommraus, F., Fischer, L. K., and Kowarik, I. (2017). Drastic site-preparation is key for the successful reintroduction of the endangered grassland species *Jurinea cyanoides*. *Biol. Conserv.* 214, 88–100. doi: 10.1016/j.biocon.2017.07.036

Vera, F. W. M. (2000). *Grazing Ecology and Forest History*. Oxon: CABI Publishing.

Wilson, J. B., Peet, R. K., Dengler, J., and Pärtel, M. (2012). Plant species richness: the world records. *J. Veg. Sci.* 23, 796–802. doi: 10.1111/j.1654-1103.2012.01400.x

Ziegenspeck, H. (1936). “Orchidaceae” in *Lebensgeschichte der Blütenpflanzen Mitteleuropas*. eds. O. Kirchner, E. Loew and C. Schröter (Stuttgart: Verlagsbuchhandlung Eugen Ulmer)

Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 27 January 2023
DOI 10.3389/fevo.2023.995133

OPEN ACCESS

EDITED BY
Péter Török,
University of Debrecen,
Hungary

REVIEWED BY
Katherina Ng,
Australian National University,
Australia
Judit Sonkoly,
University of Debrecen,
Hungary

*CORRESPONDENCE
Sophie P. Ewert
✉ Sophie.ewert@web.de

SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 15 July 2022
ACCEPTED 10 January 2023
PUBLISHED 27 January 2023

CITATION
Ewert SP, Knörnschild M, Jung K and Frommolt K-H (2023) Structurally rich dry grasslands – Potential stepping stones for bats in open farmland.
Front. Ecol. Evol. 11:995133.
doi: 10.3389/fevo.2023.995133

Structurally rich dry grasslands – Potential stepping stones for bats in open farmland

Sophie P. Ewert[1,2]*, Mirjam Knörnschild[1,2,3], Kirsten Jung[4] and Karl-Heinz Frommolt[1]

[1]Department of Evolutionary Diversity Dynamics, Museum für Naturkunde – Leibniz Institute for Evolution and Biodiversity Science, Berlin, Germany, [2]Animal Behavior Lab, Department of Biology, Chemistry, and Pharmacy, Institute of Biology, Freie Universität Berlin, Berlin, Germany, [3]Deutsche Fledermauswarte e.V., Berlin, Germany, [4]Evolutionary Ecology and Conservation Genomics, Ulm University, Ulm, Baden-Württemberg, Germany

Agricultural intensification has caused decrease and fragmentation of European semi-natural dry grasslands. While a high biodiversity value of dry grasslands is acknowledged for plants and insects, locally and on landscape level, their relevance for mobile species, such as bats, is unknown. Here we investigate the use of dry grassland fragments by bats in an agriculturally intensified region in Germany and evaluate local and landscape factors influencing bat activity and assemblages. Specifically, we predicted that a combination of local dry grassland structural richness and landscape features as well as their interactions affect bat activity and foraging above dry grasslands. We also expected that these features influence compositions of local bat assemblages. We repeatedly sampled at 12 dry grassland plots with acoustic monitoring and assessed activity and foraging of bat species/sonotypes, which we grouped into guilds known for foraging in open land, at vegetation edges and in narrow spaces. We determined structural richness of the dry grassland plots in field and derived landscape features from digital landscape data. A relatively high proportion of bat species/sonotypes used dry grasslands regularly. The edge space foragers responded positively to higher local structural richness. Their dry grassland use increased when surrounding forests and woody features were less available, but they foraged more on dry grasslands closer to water bodies. Narrow space bat activity on dry grasslands decreased with less landscape connectivity. Open and narrow space foragers responded to local structural richness only in landscape context. For all bat guilds we found increased use of structurally richer dry grasslands when there was more open farmland in the surroundings. This was also the case for edge space foragers, when landscapes were more homogeneous. Lastly, with increasing structural richness, bat assemblages were more dominated by edge space foragers. We show the importance of European dry grassland fragments for the highly mobile group of bats under certain local structural and landscape compositional conditions. Our results underline the value of heterogeneous dry grassland fragments as potential stepping stones in intensively used farmland areas and contribute to evidence based decision making in dry grassland management and bat conservation.

KEYWORDS

dry grasslands, bats (*Chiroptera*), agricultural intensification, landscape, structural richness, acoustic monitoring, habitat fragments

1. Introduction

The agricultural intensification and industrialization, starting in the second half of the 20th century, have led to significant changes in land use and ecosystems worldwide. Both belong to the major suspected causes for global biodiversity loss (Díaz et al., 2019; Raven and Wagner, 2021) and population declines of various plant, insect and animal taxa (Burns et al., 2016; Young et al., 2016). Among the drivers of these detrimental effects are homogenization and simplification of landscapes, increased patch sizes of agricultural fields, reduced landscape structures (such as hedgerows) and loss of natural and semi-natural habitats (Matson et al., 1997; Stoate et al., 2001; Tilman et al., 2001; Jongman, 2002).

Semi-natural dry grasslands are open habitats, dominated by herbaceous (vascular) plants, mosses and lichens with occasional heterogeneous shrub and tree growth. In central Europe they have been significantly reduced during the 20th century, mainly due to land conversion, abandonment and forest encroachment, leaving small remnants fragmented in agricultural areas (Poschlod and Wallis de Vries, 2002; Habel et al., 2013; Vrahnakis et al., 2013; Leuschner and Ellenberg, 2017). Dry grasslands are known for their extremely high biodiversity in special plant communities, resulting from extremely nutrient poor, dry and sun exposed local conditions (Dengler et al., 2012; Wilson et al., 2012), and a rich insect fauna (Wallis de Vries and Van Swaay, 2009; Rasran et al., 2018; Schuldt et al., 2019). Therefore, they are now protected under the EU Habitats Directive (Council Directive 92/43/EEC). Conservation management targets these characteristic dry grassland plant species communities, by aiming to reduce nutrient inflow from agriculture (e.g., buffer zones) and by the application of grazing regimes, to reduce succession of shrubs and trees (Vrahnakis et al., 2013; Leuschner and Ellenberg, 2017). Especially smaller dry grassland fragments surrounded by intensively managed land are at greatest risk of further habitat degradation and loss of associated organisms due to external environmental influences (Butaye et al., 2005; Hodgson et al., 2005). However, exactly those small fragments have shown a great importance for species richness and communities of plants (Deák et al., 2020) and insects (Tscharntke et al., 2002; Rösch et al., 2015). Such positive effects on biodiversity are known to reach beyond their borders, affecting the species communities of the surrounding landscape. Lindborg et al. (2014) for example found increased plant richness on green islands in agricultural landscapes when dry grasslands were present in the vicinity. Their presence itself, as additional habitats in crop-dominated land, facilitates occurrence of various organisms and their exchange with adjacent landscape. This has been shown for insect communities (Tscharntke et al., 2002; Duelli and Obrist, 2003) and multiple taxa of insects and plants (Zulka et al., 2014). Dry grassland fragments can thus improve overall biodiversity of the landscape by providing local suitable conditions for specific species communities, which at the same time are influenced by the surrounding landscape. While research on plants and insects on dry grasslands has been plentiful so far, other more mobile organisms from higher trophic taxa have barely been taken into account. Therefore, researchers have recommended investigating the value of dry grasslands and their management regimes for other species and highlighted the importance of multi-scale approaches including local as well as surrounding landscape factors (Söderström et al., 2001; Zulka et al., 2014; Loos et al., 2021).

European bats are highly mobile insectivorous animals, protected by the EU Habitats Directive (Council Directive 92/43/EEC). Consequences of agricultural intensification, such as habitat and connectivity losses, are among the major drivers of bat population declines (Browning et al., 2021). While mainly associated with forests and partly urban areas for roosting, bats utilize a variety of habitat types during their nightly foraging trips of several kilometers (Dietz and Kiefer, 2016). Their habitat use is thereby not only directed by insect food availability, but also by suitability of flight pathways (Kusch et al., 2004; Müller et al., 2012). The latter is defined for each bat species by their functional traits (e.g., echolocation system and wing morphology) which determine the abilities to navigate, maneuver and forage in different degrees of vegetation clutter (Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987). Based on that, bat species can be categorized into three functional guilds related to their preferred commuting and foraging habitat of open space, edge structures (such as forest edges, hedgerows) or narrow space (for example inside forests; Fenton, 1990; Schnitzler and Kalko, 2001). Local clutter conditions, determined by the structural compositions of the surroundings, can thus facilitate or hinder movement of bats from functional guilds depending on their requirements. The composition of vegetation structures, such as shrubs and trees, has shown to be of influence on bat species occurrence and activity in studies in forest systems. These studies have found species-specific activity responses of bats to various horizontal and vertical structural forest stand features (Jung et al., 2012; Froidevaux et al., 2016; Blakey et al., 2017). Highest bat activity and species diversity have been recorded in heterogeneous forest plots with intermediate vegetation clutter on different height levels, offering various structural niches (Adams and Law, 2011; Hanspach et al., 2012; Jung et al., 2012; Starik et al., 2018). Hence, on dry grasslands, one would expect a positive influence of local heterogeneous shrub and tree encroachment on bat occurrence and activity. However, dry grassland fragments, especially in open and homogeneous agricultural areas, might often be isolated from other main habitats for roosting and foraging or from structural landscape features, such as hedgerows, that offer connectivity networks as sonar guidelines, with higher prey availability and as possible protection from predators (Verboom and Huitema, 1997). When commuting through these kind of landscapes, open habitat adapted bat species do not face major constraints, while others, which are adapted to commuting and hunting closer to edges or cluttered vegetation, are likely to reach a limit in how far they move through open areas (Frey-Ehrenbold et al., 2013). These sensory constrains and habitat associations underline the necessity of a multi-scale approach when investigating the use of dry grasslands by bats embedded in open agricultural landscapes. After all, while local dry grassland conditions can offer abundant resources for commuting and foraging, the features of the surrounding landscape predominantly determine the accessibility of dry grassland fragments (Boughey et al., 2011b; Kalda et al., 2015a,b; Froidevaux et al., 2022).

A landscape should enable dispersal and support of a diverse species pool by providing a variety of resources in non-crop habitats, which is however usually limited in agriculturally intensified regions (Tscharntke et al., 2005). It is therefore crucial to understand how remaining (semi-) natural habitat fragments in farmland, such as dry grassland systems, affect endangered species. The value of dry grassland fragments for highly mobile protected bats is however still unknown. Identifying which conditions at local and landscape scales are important for bats to occur and forage on dry grasslands, adds evidence to the biodiversity value of dry grassland fragments and contributes to informed decision making for dry grassland management and bat conservation efforts. Our study therefore focuses on investigating the use of dry grassland fragments by bats in context of features of local and landscape scales as well as their interactions in an agriculturally rich region in Germany. We described the local conditions of dry grasslands with structural richness provided by shrubs and trees. In addition, we evaluated features describing openness, homogeneity and connectivity of the surrounding landscape as well as the availability of other main habitat types associated with foraging and roosting of bat species. We hypothesized that a combination of local and landscape effects as well as their interactions determines the use of dry grasslands by bats. Specifically, we expected that higher local structural richness on dry grassland fragments would increase bat activity and foraging [as in forests in Jung et al. (2012)]. Furthermore, we predicted effects of landscape features [as in Heim et al. (2015)] on use of dry grasslands by bats, and anticipated particularly the edge and narrow space adapted bats to show greater accessibility limitations (Frey-Ehrenbold et al., 2013). Most importantly however, we expected that landscape features would influence the correlation of bat activity with local structural richness [such as Froidevaux et al. (2022)]. Lastly, we predicted a dominance shift of functional bat guilds in bat assemblages above dry grasslands with changes in dry grassland and landscape features [given their different habitat preferences as in Schnitzler and Kalko (2001)].

2. Materials and methods

2.1. Study region

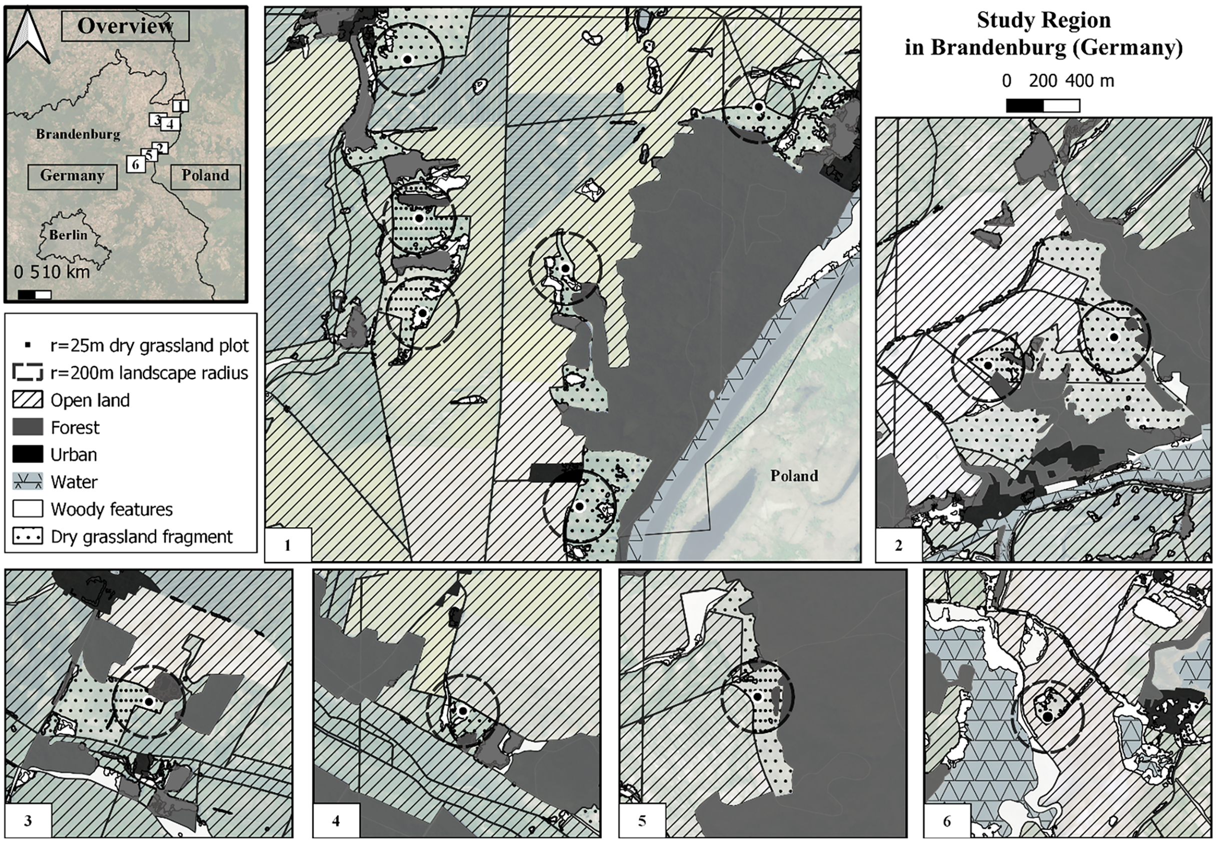
This study has been conducted on designated protected dry grassland fragments (Figure 1) in the study region North-East Brandenburg in Northeastern Germany. Brandenburg is characterized by a comparatively low human population density. It is a young glacial landscape with many wetlands. The district Uckermark (Statistical Office Berlin-Brandenburg, 2020), where the majority of dry grassland fragments from this study are located, consists of roughly 60 percent of agricultural land (mainly used for cereal grains and a low proportion for pastures). About a quarter of the landscape is covered by forest, predominantly coniferous forests, followed by deciduous and a few floodplain forests. Although there are some fragmented forest patches, the majority of forests in the study region are rather continuous and extensive. This also means that in-between, large areas are covered by open land. The dry grassland fragments are typically rather small (in this study: min. 2 ha, max. 41 ha, mean 16 ha) and sometimes long and narrow, as they have been pushed to margins of agriculturally unusable land (e.g., slopes). They are found on dry, nutrient poor and sun exposed locations, reaching sub-continental extreme microclimatic conditions. The prominent type of management to reduce shrub and tree encroachment is grazing by sheep for a short period once a year. Brandenburg carries great conservation responsibility, containing Germany's largest proportion of xeric sand calcareous and sub-pannonic steppic grassland (Landesamt für Umwelt Brandenburg, 2022). The latter type is dominating in the study region and is characterized by an open landscape with a high number of vascular plants, mosses and lichens. The dry grassland fragments chosen for this study showed a range of cover of woody structures (e.g., shrubs, small trees) from none up to 30%. These conditions are described as excellent to mediocre, respectively, in conservation value (Landesamt für Umwelt Brandenburg, 2022) and underline the heterogeneity of dry grasslands. This is especially relevant when seen in comparison to other grassland types (pastures and meadows) in the surroundings, which are mainly used for cattle grazing and are usually very homogeneous and lacking structural richness. Our dry grassland fragments differed not only in structural compositions but also in embedment in the landscape (regarding for example distances to other habitats).

2.2. Acoustic monitoring

We used Batlogger (Batlogger A, Elekon AG, Luzern, Switzerland) for repeated passive acoustic monitoring to assess bat activity above dry grassland fragments. Batlogger microphones were mounted on poles in the center of 12 designated study plots, which were intended for vegetation assessment (see 2.4), two meters above the ground and at least two meters away from larger trees to avoid strong echoes. We mounted one Batlogger per dry grassland fragment due to their small sizes, except on two larger fragments, where we used two plots each (see Figure 1). The locations of the plots were chosen in field depending on local structural conditions to cover the wide range of structural compositions. Their centers, hence Batlogger locations, were at least 500 m away from each other to prevent spatial autocorrelation and at least 30 m away from adjacent habitat types (e.g., crop and forest) to reduce edge effects. Note, however, that effects of other habitats were accounted for in the following landscape analysis. Each Batlogger sampled four nights (except at one plot, which was sampled three times due to access restrictions) throughout the main bat activity season (five plots in 2019, seven plots in 2020). Two sampling nights took place between June – July and (one) two between August – October. This resulted in 47 sampling nights. Recording started around 30 min before sunset and continued until approximately 30 min after sunrise. For triggering of sound recordings, we used the Crest Advanced setting that ensured the recording of quiet bat calls and reduced sensitivity to disturbing noise (Supplementary Datasheet S1). Sampling was avoided in nights with prolonged rainfall, high wind speed (>BFT 4; Martin et al., 2017) and low temperatures <8°C. We also avoided recording during sheep and cattle grazing, as movement of cattle can potentially increase bat activity (Downs and Sanderson, 2010; Ancillotto et al., 2017).

2.3. Bat identification and activity

We performed semi-automatic call analysis in the program Batexplorer 2.1 (Elekon AG, Luzern, Switzerland) to assign recordings with bat echolocation calls to species level or to an identifiable sonotype (groups of bat species with very similar call structures). This was based on a self-created list of identification criteria (Supplementary Table S1) derived from various sources (Parsons and Jones, 2000; Russo and Jones, 2002; Pfalzer and Kusch, 2003; Obrist et al., 2004; Skiba, 2009; Barataud, 2020). All recordings were checked by hand to reduce misidentification (Russo and Voigt, 2016). We analyzed recordings with sequences containing two or more recognizable bat echolocation signals. Non-identifiable bat calls were omitted from further analysis. We also identified recordings with one or more feeding buzzes (distinct phases with rapid, steep calls with a high frequency bandwidth) as indications for insect prey encounter and therefore foraging activity. We grouped all bat species/sonotypes into three functional bat guilds by similarities in eco-morphological adaptations and in their preferred vegetation clutter conditions of habitats (Schnitzler and Kalko, 2001; Zeale et al., 2012; Dietz and Kiefer, 2016; Barataud, 2020): (i) open space guild (open



Map created with QGIS 3.16 in Dec. 2022, by S.P.Ewert; Basemap: ESRI Satellite (ArcGIS/World_Imagery); Spatial data: ATKIS-Basis-DLM © GeoBasis-DE/LGB, dl-de/by-2-0, 2021; SWF 2015 © European Union, Copernicus Land monitoring Service, 2020

FIGURE 1
Map of study region in Brandenburg in Germany with the landscape types, locations of the dry grassland plots (*n*=12) and the landscape radii for analysis.

habitats, high and fast flying bats with low maneuverability, narrowband and low-frequency echolocation calls), (ii) edge space guild (uses background cluttered habitats, such as forest edges, but is flexible in open and clutter; bats with intermediate speed and maneuverability; medium frequency echolocation calls with steep broadband and narrowband components), and (iii) narrow space guild (uses cluttered habitats close to vegetation; bats with low flight speed and high maneuverability; steep broadband echolocation calls). For a measure of habitat use of each guild, we took bat activity, which was defined by the recorded number of minutes of one night per plot, in which we found at least one echolocation recording (with a minimum of two calls) of the respective bat guild (Miller, 2001). We did the same for recordings including feeding buzzes as a measure of foraging activity. Feeding buzzes were not sampled for bats from the narrow space guild, as they mainly use gleaning and passive listening to find insects, instead of feeding buzzes (Entwistle et al., 1996; Schnitzler and Kalko, 2001).

2.4. Local structural richness of dry grasslands

From forest systems it is known, that higher three-dimensional structural heterogeneity supports a higher number of bat species and their activity (Adams and Law, 2011; Hanspach et al., 2012; Jung et al., 2012; Starik et al., 2018). We thus used an index that describes vertical as well as horizontal structural richness of the dry grassland plots. Therefore, we used the foliage height diversity index (FHD), which was originally proposed by MacArthur and MacArthur (1961) as a description of heterogeneity in vegetation layer coverage and to predict bird species richness. It was later adapted in other studies for birds (Wood et al., 2013) and for bat occurrence in forest systems (Froidevaux et al., 2016). For calculating the FHD as a measure of structural richness, we applied the Shannon-Wiener Index [in R 4.0.3. by R Core Team (2020); package: vegan, Oksanen et al., 2020; function: diversity], as proposed in Froidevaux et al., (2016). For that, we used cover values of foliage height classes: ground 0–1 m, shrub 1–5 m, tree low 5–10 m, tree high >10 m. To assess vegetation cover per height class, we did in-field vegetation sampling of the study plots within a 25 m radius around the Batlogger locations. We identified the positions and average heights of woody vegetation structures (shrubs and trees) that were taller than 0.5 m and drew the vegetation layout true to scale on a grid map. For height estimation of larger trees, we used a tree-height-measuring app (App: "Baumhöhenmesser," Foresttools, Göttingen, Germany). In the following, looking at the drawn map and knowing the heights of the woody vegetation, we estimated the amount of square meters covered by woody vegetation in each defined height class and then calculated the percentage of the total plot covered by each height class. The FHD correlated positively with other descriptives of the dry grassland plots (cover values of height classes and total foliage cover) with Pearson coefficients from 0.8 and higher [in R 4.0.3. by R Core Team (2020), package: Ggally, by

Schloerke et al. (2021), function “ggcorr”]. We therefore considered the FHD as an appropriate proxy for the description of local structural richness.

2.5. Landscape features

For the landscape analysis, we considered landscape features that are of known relevance for bats and would aid in describing the landscape in terms of openness, homogeneity, connectivity and availability of resources for bats. We thus included forests and urban areas, as important roosting and foraging habitats (e.g., Dietz and Kiefer 2016), as well as water bodies, offering valuable foraging sites especially in open land (e.g., Heim et al., 2018). We also considered woody features (e.g., hedges, tree lines and small woodland patches), as important connectivity features for many bat species using them for sonar guidance, protection and foraging (e.g., Verboom and Huitema, 1997). In addition, we included open land, consisting of pastures and predominantly cropland, known to negatively affect bat activity (e.g., Put et al., 2019). Lastly, we considered patchiness of the landscape as a measure for homogeneity. A homogeneous landscape, with less patches, can be detrimental for some bat species (e.g., Mendes et al., 2017) as it provides less heterogeneous niches and edge habitats for commuting and hunting (Ethier and Fahrig, 2011). The landscape features and the used measurements can be found in Table 1. To derive measurements of landscape features we performed landscape analysis (Supplementary image S1) within a 200 m radius around the Batlogger position using the geographic information system QGIS 3.16.3 (QGIS Development Team, 2021). We adapted a 200 m radius, as this distance has already shown significant effects of landscape features on bat activity and species composition, for example in Heim et al. (2015). We used layers with detailed landscape data (spatial data: ATKIS-Basis-DLM, source: ©GeoBasis-DE/LGB, dl-de/by-2-0, 2021). The categories of the landscape layers were then reclassified into broader categories: urban area, water body, forest and open land (pastures + cropland; Supplementary Table S2) and used for subsequent spatial analysis in QGIS. Furthermore, we used a high-resolution layer of woody features, which consisted of patchy, linear and additional woody structures, such as hedges and tree lines (spatial data: Small Woody Features 2015, source: ©European Union, Copernicus Land monitoring Service, 2020). Woody features directly connected to forest were reclassified as forest when they were protruding not more than 5 m from the forest. Further, we corrected major misclassification errors in landscape categorizations by checking in the field. We then calculated within the 200 m radius the cover of forest, open land (pastures + cropland) and woody features in percentage. Additionally, we counted the number of landscape patches regardless of their types within the 200 m radius. Separate patches could be for example landscape features distinct from their surroundings (e.g., forest or woody patches) or when divided by paths or roads (e.g., separate fields of crop or pastures). Lastly, we measured the linear distances from the Batlogger locations to the nearest urban areas, woody features and water bodies. We checked all variables for collinearity and found Pearson coefficients only between −0.6 and 0.6, indicating no notable correlations [in R 4.0.3. by R Core Team (2020), package: Ggally, by Schloerke et al. (2021), function “ggcorr”].

2.6. Statistical analysis

The following statistical analyses were performed in R (version 4.0.3, R Core Team, 2020).

2.6.1. Modeling responses to local and landscape features

To assess whether bat activity and foraging of each functional guild responded to increased structural richness (FHD) of dry grassland

TABLE 1 Final choice of local and landscape features and their abbreviations used for further analysis.

Local & Landscape features	Abbreviation	Relevance for bats found in:
Measurements		
Dry grassland ($r = 25$ m)		
Foliage height diversity (structural richness)	FHD	Froidevaux et al. (2016)
Landscape ($r = 200$ m)		
Forest: *Percentage cover*	For	Fuentes-Montemayor et al. (2013); Heim et al. (2015, 2018); Kalda et al. (2015b); Treitler et al. (2016)
Open land (pastures + cropland): *Percentage cover*	Open	Put et al. (2019)
Patches: *Counted number*	Patch	Mendes et al. (2017)
Urban area: **Distance*	DistUrb	Fuentes-Montemayor et al. (2013); Heim et al. (2015)
Water bodies: **Distance*	DistWat	Downs and Racey (2006); Stahlschmidt et al. (2012); Fuentes-Montemayor et al. (2013); Heim et al. (2015, 2018); Mendes et al. (2017)
Woody features: *Percentage cover* **Distance*	Wood DistWood	Verboom and Huitema (1997); Downs and Racey (2006); Boughey et al. (2011b); Frey-Ehrenbold et al. (2013); Kelm et al. (2014); Heim et al. (2015); Kalda et al. (2015a); Toffoli (2016)

Right column shows examples of literature where the landscape variable has shown a relevance for bats. *Distances were also measured when reaching outside of the 200 m radius.

TABLE 2 List of identified species/sonotypes in their associated functional guilds and the accumulated number of activity/foraging minutes based on 47 sampling nights.

Species/sonotypes	Total numbers of minutes	
	Activity	Foraging
Open space guild		
Eptesicus serotinus	177	3
Nyctaloid	424	23
Nyctalus leisleri	139	6
Nyctalus noctula	2,278	215
Edge space guild		
Barbastella barbastellus	2	0
Pipistrellus high	177	15
Pipistrellus low	168	7
Pipistrellus nathusii	1,180	49
Pipistrellus pipistrellus	784	56
Pipistrellus pygmaeus	923	107
Narrow space guild		
Myotis myotis	11	0
Myotis spp.	215	2
Plecotus spp.	39	0

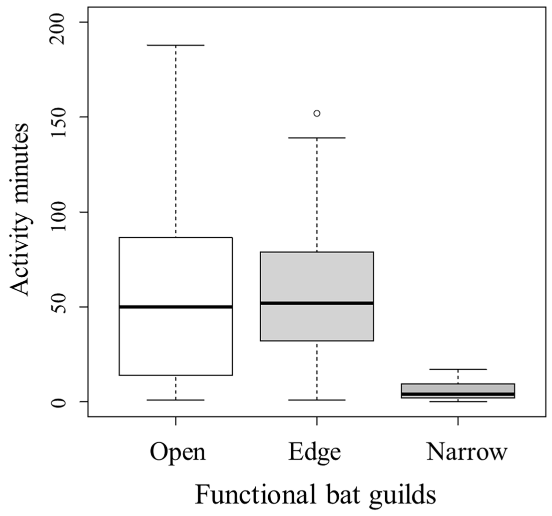


FIGURE 2
Nightly activity minutes of bat guilds (open, edge, and narrow space) counted during 47 nights on 12 dry grassland plots.

plots, as well as to landscape features and/or the interaction of both, we applied information-theoretic model selection with generalized linear mixed models (R package: glmmTMB, Brooks et al., 2017). This approach was chosen over an *a priori* set of candidate models, as we expected all predictors to have effects on bat activity and foraging to some extent (see "Landscape features") and it allowed us to find the most explanatory set of predictors (Bolker et al., 2009; Frey-Ehrenbold et al., 2013; Kalda et al., 2015a; Mendes et al., 2017). We used a negative binomial link due to overdispersion. FHD and landscape features were taken as fixed effects and mean nightly temperatures and the IDs of the dry grassland plots were taken as random effects. All predictors were scaled using the scale function (Becker et al., 1988). We conducted stepwise forward selection while keeping FHD in every model and in a stepwise manner adding landscape features as main effects and then as interaction terms with FHD, looking for the model with the best AIC value indicating the most parsimonious model (Supplementary Table S3). We chose forward selection for the following reasons: first, to reduce the risk of convergence issues due to overly complex models; secondly, to mirror our research questions approach, by initially testing for effects of dry grassland structural richness and then adding features from the broader landscape scale. Model selection was performed for all guilds and their activity as well as their foraging activity (except for the narrow space guild).

2.6.2. Functional composition of bat assemblages

From the cumulative total bat activity of all three functional guilds per night and plot, we calculated the percentages of nightly activity per guild. From there we derived the median activity per dry grassland plot for each guild. This made the dry grassland plots comparable in their proportions of functional guild activity counts. To investigate the proportional differences on dry grassland fragments we performed non-metric multidimensional scaling (NMDS in R package: vegan; function: metaNMDS, Oksanen et al., 2020). We then applied environmental fitting of the dry grassland and landscape features onto the ordination (R package: vegan, Oksanen et al., 2020; function: envfit) to investigate their potential to explain proportional changes of bat assemblages between dry grassland plots. Results were supported by using permutational multivariate analysis of variance [PERMANOVA in R package: vegan, Oksanen et al., 2020; function: adonis, based on Anderson (2001)].

3. Results

3.1. General results

At 12 dry grassland plots, during a total of 47 sampling nights, we found 13 bat species/sonotypes that we had grouped into the three functional guilds (Table 2) and 5,488 minutes with echolocation activity of one or more bat species/sonotypes. Bats were active during 19% of the total recording time (28,560 min) in which one or more bats could have been recorded. Per night at a plot, we counted on average almost 2 hours of bat activity (mean: 117 min). Eight percent of activity minutes contained feeding buzzes of one or more bat species/sonotypes from the open and edge space guild, indicating foraging activity. Nightly activity (Figure 2) mainly consisted of the open and the edge space guild. Activity of bats from the narrow space guild was generally much lower. The activity of the open space guild was dominated by *Nyctalus noctula* (75%). The edge space guild showed shared dominances of *Pipistrellus nathusii* (37%), *Pipistrellus pygmaeus* (29%) and *Pipistrellus pipistrellus* (23%). Lastly, the *Myotis* group (81%) formed the largest part of the narrow space guild. Each dry grassland plot was visited by an average of 11 bat species/sonotypes throughout the year, with a nightly mean of eight, indicating a regular use by a high proportion of the recorded bat species/sonotypes. Additionally, we recorded at least three species/sonotypes of higher conservation concerns according to the German Red List (Meinig et al., 2020): *Eptesicus serotinus* (threatened) on 11 plots, *Plecotus* spp. (threatened) on 9 plots and *Barbastella barbastellus* (highly threatened) on one plot.

3.2. Responses to local and landscape features

Model selections revealed that a combination of both local (plot level) and landscape scales, and especially their interactions, is important for explaining activity (Table 3) and foraging (Table 4) of bat functional guilds at the dry grasslands.

Bats from the edge space guild showed strong associations with local structural conditions. Their activity generally increased significantly with higher structural richness (FHD), regardless of landscape interactions. We also found significant additive effects of features on the landscape scale on edge space bats. They foraged more above dry grassland plots when water bodies were closer by, indicating associations with other main habitat types. However, they were more active on dry grasslands plots when there was less forest in the surrounding and their foraging activity increased with greater distances to woody features (Figure 3), suggesting an increased use of dry grasslands when other habitat sources are less available. Activity of bats from the narrow space guild decreased at dry grassland plots further away from woody features (Figure 3), showing a stronger accessibility limitation.

For all functional guilds, we found significant interaction effects of local structural richness in combination with landscape features describing openness, homogeneity and availability of main habitats. The activity of open and narrow space bats (Table 3) as well as the foraging activity of open and edge space bats (Table 4) increased above dry grassland plots with more local structural richness, when they were surrounded by a higher percentage of open land (pastures and cropland). In less open landscapes, structural richness was less important for the open space guild (Figures 4A,B) and the narrow space guild (Figure 4D), while in the foraging activity of the edge space guild, the effect was still positive but less prominent (Figure 4C). Additionally, edge space bat foraging increased on structurally richer dry grassland plots, when the number of patches in the surrounding decreased (Figure 5). Lastly, structurally richer dry grassland plots were used more by open space bats (increase in activity and foraging) and foraged more by edge space bats, when urban areas were further away.

3.3. Functional compositions of bat assemblages

Non-metric multidimensional scaling separated dry grassland plots based on the median percentage of nightly activities of the function guilds (Figure 6; NMDS: $k = 2$, stress = 0.006, perm = 999, Supplementary Table S4). Environmental fitting including local and landscape features showed that the structural richness (FHD) significantly explained separation of dry grassland plots along the y-axis ($r^2 = 0.68$, $p = 0.010$). Especially the activity proportion of edge space guild increased with higher structural richness. This was supported by results of the PERMANOVA (FHD: $r^2 = 0.54$, $p = 0.022$, Supplementary Table S5) and suggests that structural composition of dry grassland fragments influences assemblage composition of bats. Landscape features did not show significant effects.

TABLE 3 Results of bat guild activity predictions from the forward selections of generalized linear mixed models (GLMM) with negative binomial links based on selection of the best Akaike's information criterion (AIC).

GLMM model	Parameter	Estimate	Standard error	*Z* value	Pr(>|z|)
Open space guild activity	(Intercept)	3.1427	0.2767	11.360	***
AIC: 468.1	FHD	−0.1365	0.1291	−1.057	n.s.
	Open	0.1408	0.1343	1.048	n.s.
	DistUrb	0.6843	0.1945	3.518	***
	Wood	−0.3267	0.1907	−1.713	.
	FHD*Open (Figure 4)	1.1413	0.2499	4.567	***
	FHD*DistUrb	0.9694	0.2784	3.482	***
Edge space guild activity	(Intercept)	3.8142	0.2088	18.264	***
AIC: 459.6	FHD	0.3939	0.1656	2.379	*
	For	−0.5750	0.2398	−2.398	*
Narrow space guild activity	(Intercept)	0.88980	0.27875	3.192	**
AIC: 257.4	FHD	0.40062	0.28511	1.405	n.s.
	Open	0.14072	0.15188	0.927	n.s.
	DistWood (Figure 3)	−0.76303	0.28555	−2.672	**
	Patch	0.05497	0.26329	0.209	n.s.
	FHD*Patch (Figure 4)	1.10942	0.31057	3.572	***
	FHD*Patch	0.64107	0.37177	1.724	.

Listed are the final models for activities of open, edge and narrow space guilds based on 47 nights at 12 grassland dry grassland plots and the habitat and landscape features (refer to Table 1). Positive estimate values (+) indicate positive effects and negative values (−) negative effects. n.s., non-significant; ‘.’$p < 0.1$; * $p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 4 Results of bat guild foraging activity predictions from the forward selections of generalized linear mixed models (GLMM) with negative binomial links based on selection of the best Akaike's information criterion (AIC).

GLMM model	Parameter	Estimate	Standard error	*Z* value	Pr(>|z|)
Open space guild foraging	(Intercept)	0.76786	0.42613	1.802	.
AIC:252.1	FHD	−0.04104	0.35316	−0.116	n.s.
	Open	0.59091	0.29060	2.033	*
	DistUrb	0.74269	0.35610	2.086	*
	FHD*Open (Figure 4)	1.47709	0.60054	2.460	*
	FHD*DistUrb	1.10715	0.55029	2.012	*
Edge space guild foraging	(Intercept)	−0.4091	2.1632	−0.189	n.s.
AIC: 221.8	FHD	−2.3540	2.3254	−1.012	n.s.
	DistWat	−3.4008	0.9687	−3.511	***
	DistUrb	0.9301	0.9068	1.026	n.s.
	Open	1.2013	0.2157	5.568	***
	Patch	−3.9534	0.7601	−5.201	***
	DistWood (Figure 3)	2.3413	1.1500	2.036	*
	FHD*DistUrb	3.4652	1.3265	2.612	**
	FHD*Open (Figure 4)	4.0477	0.7864	5.147	***
	FHD*Patch (Figure 5)	−8.9213	1.8411	−4.846	***
	FHD*DistWood	1.9979	1.8752	1.065	n.s.

Listed are the final models for foraging activities of open, edge and narrow space guilds based on 47 nights at 12 grassland dry grassland plots and the habitat and landscape features (refer to Table 1). Positive estimate values (+) indicate positive effects and negative values (−) negative effects. n.s., non-significant; $.p < 0.1$; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

4. Discussion

Loss of (semi-) natural habitats and ecosystem changes are consequences of agricultural intensification, which is globally identified as the major cause of the current biodiversity crisis (Burns et al., 2016; Young et al., 2016; Díaz et al., 2019; Raven and Wagner, 2021). The presence of non-crop habitats in intensified agro-ecosystems is crucial to enable occurrence and dispersal of a variety of organisms that are necessary to sustain a resilient species pool and associated ecosystem services (Tscharntke et al., 2005; Fischer et al., 2006). In this study we focused on fragmented, protected European semi-natural dry grasslands, which are known for a very high plant biodiversity (Dengler et al., 2012; Wilson et al., 2012) and a rich insect fauna (Wallis de Vries and Van Swaay, 2009; Rasran et al., 2018; Schuldt et al., 2019). An increased value has been particularly proven for small dry grassland fragments in agro-ecosystems, locally (Rösch et al., 2015; Deák et al., 2020) and in exchange with the surrounding landscape (Tscharntke et al., 2002; Duelli and Obrist, 2003; Lindborg et al., 2014; Zulka et al., 2014). However, their importance for highly mobile species, such as bats, is not yet understood, even though many bat species face challenges of agricultural intensification, such as habitat and connectivity losses (Browning et al., 2021) and when moving through open land (Frey-Ehrenbold et al., 2013). Revealing which local structural conditions and landscape features as well as their interactions increase commuting and foraging of bats on dry grassland fragments, is of utmost importance for bat conservation and for evidence-based decision making in management and conservation efforts of dry grasslands. Our results shed light onto the importance of dry grassland fragments for bats in a farmland-dominated region in Germany in relation to local vegetation structural richness and the availability of other important habitats. This is especially relevant in agriculturally rich regions, where other non-crop habitats are reduced (Burns et al., 2016) or more distant. We show regular visitations by a relatively high number of bat species/sonotypes throughout the year, also by species/sonotypes of higher conservation concern. Our results clearly show, that dry grassland use by bats is determined by local structural richness, the features of the surrounding landscape and the interaction of both.

4.1. Local and landscape features determine dry grassland use

As predicted, our results show additive effects of local and landscape features on dry grassland use by bats. This is in accordance with findings of other studies that highlighted the influence of landscape features at different scales on bat occurrence and their activities (Boughey et al., 2011a; Kalda et al., 2015b; Heim et al., 2017; Mendes et al., 2017).

Bats from the edge space guild appeared to be the most responsive to local conditions of dry grassland fragments. Their activity above dry grassland plots generally increased with a higher structural richness, hence more heterogeneous shrubs and trees. This is corroborated by findings of previous studies, showing that edge space bats prefer moving along structural features (Verboom and Huitema, 1997; Downs and Racey, 2006; Toffoli, 2016), specifically close to taller trees (Froidevaux et al., 2022) and it suggests that structurally richer dry grasslands have a higher value than very open ones, for edge space bats commuting in relatively open agricultural

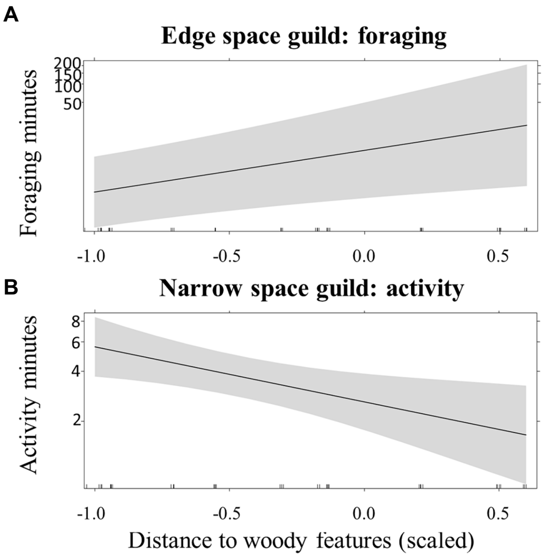


FIGURE 3
Effect plot for the predicted effects of distance to woody features (scaled) on **(A)** edge space guild foraging and **(B)** narrow space guild activity.

landscapes settings. When looking at landscape features, edge space bats were more active above dry grassland plots when less forest was available in the surrounding, and they foraged more at dry grassland plots when woody features (such as hedgerows) were further away. This is opposite to findings of other studies that show higher activity of the edge space bats (such as *Pipistrellus* species) close to forest (Davidson-Watts et al., 2006), forest edges (Heim et al., 2018) and woody features (Verboom and Huitema, 1997; Downs and Racey, 2006; Frey-Ehrenbold et al., 2013). However, the latter studies also highlight that *Pipistrellus* species can cover greater distances in open land away from favorite habitats to reach other favorable land patches. According to Kelm et al. (2014), the biggest activity drop of *Pipistrellus* species away from structural edges into open land is usually within the first 50 m. Since we show increased activity at dry grassland plots away from woody features (here average distance: 44 m) and with less surrounding forest cover, we propose that dry grassland fragments are possibly favorable habitats for bats from the edge space guild (see also Davidson-Watts et al., 2006 for *Pipistrellus* species in unimproved grasslands) and potentially fulfill an important function for commuting and foraging. These results are not supporting our expectation of accessibility limitations for edge space bats, although this could possibly become the case in regions where dry grasslands fragments are too isolated from other connecting landscape features (Frey-Ehrenbold et al., 2013). In contrast, but as expected, the narrow space guild exhibited stronger accessibility restrictions by showing reduced activity on dry grassland plots further away from woody features. This is in accordance with findings from Frey-Ehrenbold et al. (2013) showing the greatest limitations in open land for the narrow space bats of the *Myotis* and *Plecotus* groups. Therefore, isolated dry grasslands fragments might eventually fall out of their reach, if they are beyond their accessibility threshold.

Our results also highlight the importance of other habitat types in the landscape. Edge space bats foraged more on dry grassland plots closer to waterbodies. The high value of the latter for various bat species as foraging ground has been supported by other studies before (Stahlschmidt et al., 2012; Kalda et al., 2015a; Heim et al., 2018). Wetlands are also among the most preferred habitats for the edge space bat species *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Davidson-Watts et al., 2006; Dietz and Kiefer, 2016).

We did not find independent effects of local structural richness on bats from the open and the narrow space guild, but only in interaction with landscape features, indicating that for these bats surrounding conditions determine the importance of dry grasslands and their structures (discussed below). We also did not find significant effects of surrounding forest cover on bat activity and foraging in the open and narrow space guilds, even though other studies have shown positive associations of several bat species with forest proximity (Frey-Ehrenbold et al., 2013; Treitler et al., 2016) and cover (Boughey et al., 2011a; Heim et al., 2015; Mendes et al., 2017). We suppose that changes in the overall low forest cover in the 200 m radius (mean: 7%) were irrelevant for the fast flying open space bats and that the long distances (mean: 153 m) excluded many woodland dwelling narrow space species, keeping their activity low.

Our results here indicate the use of dry grasslands by bats in relation to local conditions and to availability of other main bat habitats and landscape features, which mainly affected bats from the edge and narrow space guild. While dry grassland fragments appear beneficial for commuting and foraging of bats from the edge space guild, especially when containing heterogeneous vegetation structures, narrow space bats show a dependency on higher connectivity to other structural landscape features (such as hedgerows). Our findings therefore also underline the necessity of connectivity networks and corridors in open farmland (Rosenberg et al., 1997) and the availability of other important habitats (Heim et al., 2015) to facilitate bat dispersal and provide additional resources.

4.2. Landscape setting determines the importance of local conditions for bats

We furthermore confirmed our prediction that landscape features would influence the correlations with local structural conditions and therefore determine dry grassland use. Key findings in this study are the impacts of the amount of open land (pastures and cropland) and of the number of landscape patches on the bat activity correlation with local structural richness. Bats used structurally richer dry grassland plots more for commuting and/or foraging, when the surroundings were more open (all guilds) and less patchy, hence more homogeneous (edge space guild). Both parameters, openness and homogeneity of landscapes, are of special importance, since they usually are negative consequences of agricultural intensification (Matson et al., 1997; Stoate et al., 2001; Tilman et al., 2001; Jongman, 2002; Burns et al., 2016). They very likely determine resource availability for bats in the landscape due to the following reasons: A higher amount of open farmland means the reduction of other main non-crop habitats, necessary for commuting and foraging. Similarly, homogeneous landscapes with less patches usually contain fewer crop types with reduced insect diversity (Olimpi and Philpott, 2018), and fewer habitat edges (Ethier and Fahrig, 2011). Consequently, previous studies have found decreasing bat activities of several bat species in farmland with greater amounts of open agricultural land (Put et al., 2019),

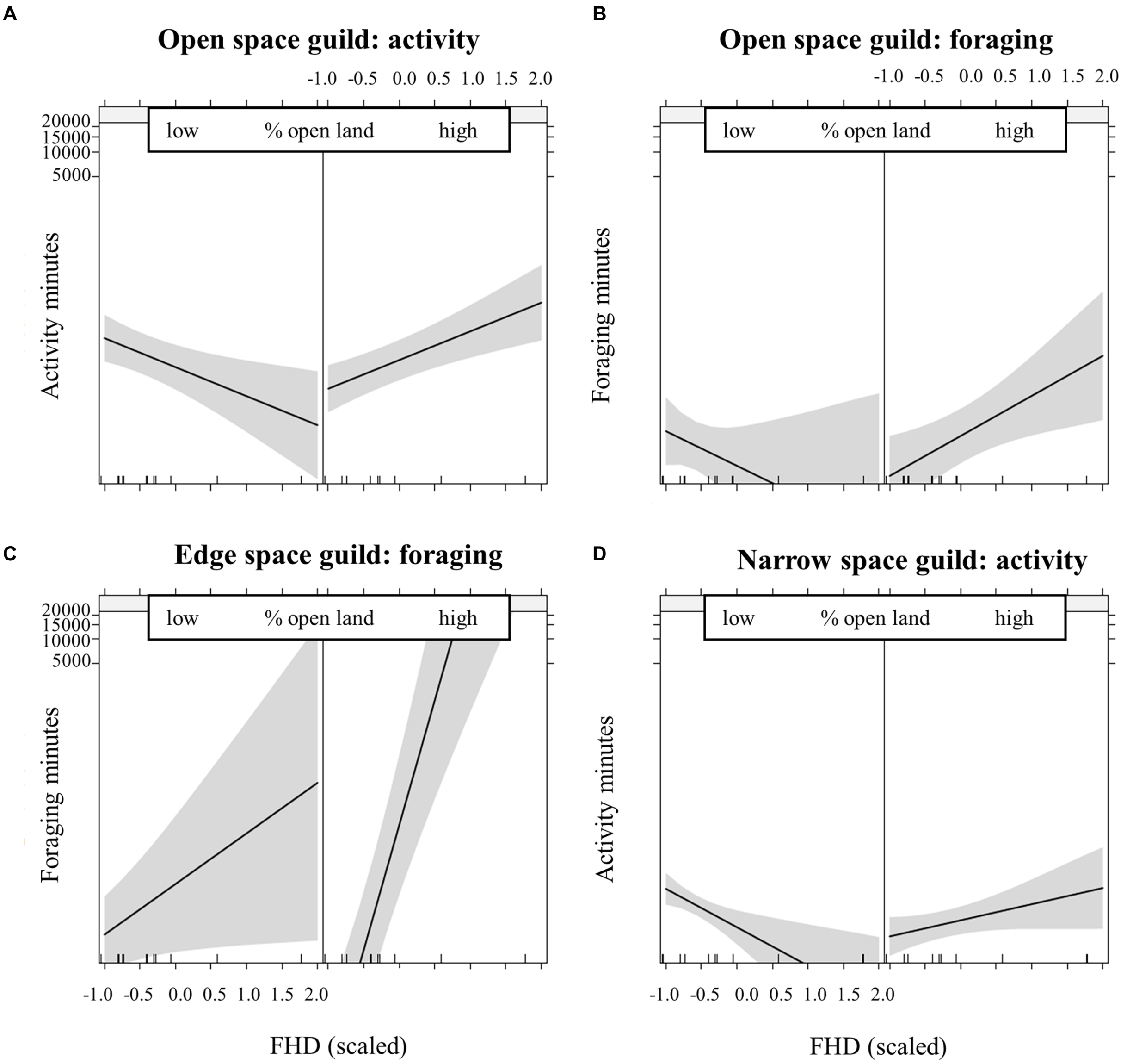


FIGURE 4
Effect plots of the interaction of the foliage height diversity (FHD, scaled) and % open land (scaled). Boxes show the predicted effects of FHD in low and high % of open land on **(A)** open space guild activity and **(B)** foraging, **(C)** edge space guild foraging, and **(D)** narrow space guild activity. Low and high values are represented by the first and third quartile of the open land data (scaled) respectively.

above improved meadows and pastures when further away from other important habitats (Heim et al., 2015; Treitler et al., 2016), and with less landscape heterogeneity (Monck-Whipp et al., 2018; Froidevaux et al., 2022). The latter especially facilitates edge bats movement (Mendes et al., 2017), which could explain why patchiness only appeared in results for the edge guild. Despite the just named known negative effects of a high amount of open farmland and heterogeneity loss, we found locally increased bat activity and foraging above dry grassland plots, when these provided higher structural richness. This is also contrary to findings of Froidevaux et al. (2022), who showed that local features become more relevant for many bat species only when the landscape is more heterogeneous. We therefore argue that the value of dry grassland fragments with diverse shrub and tree vegetation in intensive farmland increases, when the surrounding landscape becomes more unfavorable. For that, we provide the following explanations: first, the higher richness in vegetation structures can offer more niches, aiding a high variety of bat species with different sensory requirements and habitat adaptations, as it is known from forest systems (Adams and Law, 2011; Hanspach et al., 2012; Jung et al., 2012; Müller et al., 2013; Froidevaux et al., 2016; Blakey et al., 2017; Starik et al., 2018). Secondly, dry grasslands contain a rich insect fauna (Wallis de Vries and Van Swaay, 2009; Rasran et al., 2018) as potential food sources. The presence of more and denser vertical woody vegetation structures, such as trees, can further increase insect abundance (Müller et al., 2012; Fuentes-Montemayor et al., 2013), especially in agricultural regions [such as at trees and hedgerows in Grüebler et al. (2008)]. In the surrounding landscape, certain types of farmland, such as orchards or vineyards, could also provide diverse vegetation structures. Additionally, even open cropland can offer occasional plentiful insect food sources for bats. However, the latter is usually ephemeral and depending on crop

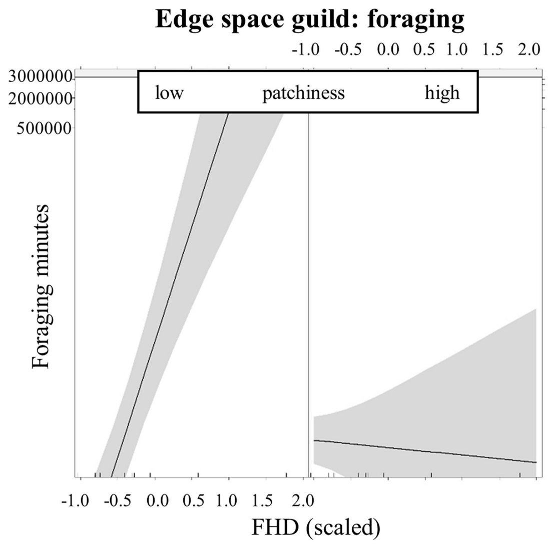


FIGURE 5
Effect plots of the interaction of the foliage height diversity (FHD, scaled) and number of patches (scaled). Boxes show the predicted effects of FHD in low and high amount of number of patches for foraging activity of the edge space guild. Low and high values are represented by the first and third quartile of the number of patches (scaled) respectively.

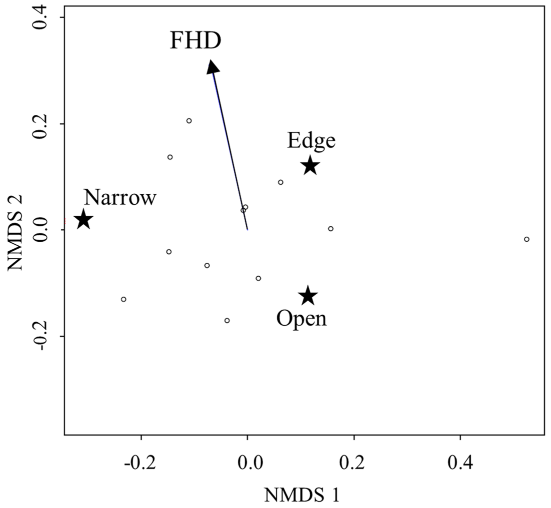


FIGURE 6
NMDS ordination plot showing the 12 dry grassland plots in $k=2$ dimensions. Placement of plots is based on Bray–Curtis dissimilarity of the activity compositions of functional bat guilds (indicated by stars). Environmental fitting was used to find explanatory variables for differences in guild composition. The significant variable (FHD) is represented as a vector.

growth and season (Heim et al., 2016, 2017; Stahlschmidt et al., 2017). Moreover, in conventional farmland, feeding on insects will lead to direct contamination of bats with pesticides (Stahlschmidt and Brühl, 2012; Stahlschmidt et al., 2017). Dry grasslands on the other hand, as semi-natural habitats, can provide stable food supplies for bats throughout the growing season (Davidai et al., 2015) and thus buffer negative effects of intensive farming practices.

We additionally found prominent interaction effects of distance to urban areas and local dry grassland conditions in the open space (activity and foraging) and edge space (foraging) guild, as their use of dry grassland plots increased with higher structural richness and with greater distance to urban areas. It is known that some species especially from open and edge space guilds regularly utilize urban areas for roosting and foraging (Jung and Threlfall, 2018) and that the proximity to anthropogenic areas can positively affect bat species occurrence and activity in grasslands nearby (Heim et al., 2015). Our study region contained mainly rural, low-density settlements, which have great potential as roosting or feeding sites (Gehrt and Chelsvig, 2004; Dixon, 2012). We therefore argue, that structurally richer dry grasslands increase in value when further away from such urban sites.

Our results here could only be obtained by investigating interaction terms of landscape and local features. As other studies suggested, effects of landscape conditions on bats at a broader scale are usually dominant over local conditions (Kalda et al., 2015a,b) but interaction terms can reveal scenarios where local features become relevant. Hence, the availability of resources for commuting and foraging provided by the surroundings determine the importance of local features. We thus conclude that in simpler and more open agricultural regions, structurally richer dry grasslands are favored by bats over less structured ones.

4.3. Functional guild assemblages shift with local features

In accordance to our prediction, we found that local structural conditions on dry grasslands change bat guild activity compositions. Our results show a proportional shift from open space to edge space guild activity with higher structural richness of plots. This again highlights the previously discussed positive affinity of edge space bats with structurally richer dry grasslands, which makes them the dominating guild, when diverse shrubs and trees are available. This corroborates the results of Davidson-Watts et al. (2006), who found that (scrubby) grasslanda are among favored habitat types for *Pipistrellus pipistrellus* and *P. pygmaeus*. We did not clearly see dominance changes along the structural richness gradient in the narrow space guild, which seems counterintuitive given their affinity to vegetation clutter. The activity of the narrow space guild however was generally low, which might have been caused by the overall open character of the landscape, and proportional changes were likely to be minor, compared to the other dominant guilds.

Heim et al. (2015) found shifts in species composition above pastures and meadows in correlation with cover of and distances to landscape features in a 200 m radius. In contrast, we did not find any explanation for shifts of bat assemblages by any landscape features. Here the limited sample size of dry grassland plots embedded in not extremely varying landscapes (given that most dry grassland fragments are located in farmland), might have obscured possible evidences.

4.4. Conclusion and further applications

To our knowledge, we show for the first time the importance of European dry grassland fragments for bats under certain local and landscape compositional conditions in an agriculturally rich region in Germany. While we confirm a constant use of dry grasslands by a high number of bat species/sonotypes throughout the season, we also show that effects of local and surrounding landscape features on bat activity and foraging vary between functional bat guilds. Nonetheless, our study emphasizes for all bat guilds the significant value of dry grasslands with increased local structural richness in unfavorable, open and homogeneous farmland. We thus argue that dry grassland fragments especially with heterogeneous shrub and tree vegetation have a high potential as stepping stones for bats in intensive agricultural regions. By that means, we underline the necessity of heterogeneity on different spatial scales for biodiversity in such landscapes (Benton et al., 2003). Our findings are of high ecological importance and conservation concern, as many bat species are facing increased challenges in agriculturally intensified regions through habitat and connectivity loss (Browning et al., 2021), but are providing important services as insect pest control (Cleveland et al., 2006; Kalka and Kalko, 2006; Kalka et al., 2008; Williams-Guillén et al., 2008). With our results, we add evidence to the high biodiversity value of European dry grassland fragments in agricultural regions, which was previously mainly shown for plants (Lindborg et al., 2014; Deák et al., 2020) and insects (Tscharntke et al., 2002; Duelli and Obrist, 2003; Rösch et al., 2015). Lastly, our findings contribute to informed decision making for dry grassland management and bat conservation.

For further applications, we highlight the importance of careful considerations before removal of heterogeneous shrub and tree vegetation on dry grassland fragments. This is contradicting the current management goals of dry grasslands, aiming to reduce shrub and tree encroachment. However, in this study we showed positive correlations of bat activity with increased structural richness, while the maximum structural richness corresponded with a rather low foliage cover of 25% (summed up cover of shrub and tree foliage), which is still equivalent to a mediocre management state for the dry grassland types found in Brandenburg, Germany (Landesamt für Umwelt Brandenburg, 2022). We thus propose considering leaving heterogeneous shrubs and trees on dry grassland fragments particularly in open, homogenous and intensively used agricultural regions, while this appears less important in heterogeneous landscapes. In line with previous studies, we highlight the need of connectivity networks (such as hedgerows) in landscapes to allow accessibility of edge and narrow space bat species (Frey-Ehrenbold et al., 2013; Heim et al., 2015). More importantly, we strongly advise to co-evaluate interactions of local and landscape features, as this leads to a better understanding of the landscape settings that determine the importance of dry grassland conditions for bats. Finally, we encourage more research on the relevance of these habitat types for single bat species, in different regions and subsequently also for other non-target organisms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because acoustic monitoring of bats is non-invasive and does not involve any animal contact. However, fieldwork and methodological approaches were approved by the State Office for the Environment and access was permitted by the nature conservation authorities of Barnim and Uckermark (districts in Brandenburg, Germany).

Author contributions

SE conceptualized and designed the study. Data collection in the field was performed by SE and K-HF. SE did the data processing and statistical analysis. The manuscript was written by SE. MK, KJ, and K-HF contributed to all previous steps through guidance and supervision. All authors further contributed to manuscript revision and read and approved the submitted version.

Funding

This research was funded as a part of the project INPEDIV (SAW-2019-ZFMK-3-INPEDIV). Open Access Funding was provided by the Freie Universität Berlin.

Acknowledgments

We thank Livia Schäffler (Zoological Research Museum Alexander Koenig, Bonn, Germany) for her contribution as project lead and coordination in INPEDIV and in acquisition of fieldwork permissions for dry grassland sites. We greatly appreciate the assistance and efforts of Artur and Anna Ewert in fieldwork. We thank the various landowners and managers as well as cattle herders and farmers for their cooperation as well as their great interests and openness toward our work. We would like to thank participants of INPEDIV for their exchange and specifically Carsten Brühl, Bodo Möseler, and Benito Schoepke for their support and input. We greatly appreciate Neil Middleton for his exchange about bat acoustics. Lastly, we acknowledge support by the Open Access Publication Initiative of Freie Universität Berlin.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.995133/full#supplementary-material

References

Adams, M. D., and Law, B. S. (2011). "A preliminary assessment of the impact of forest thinning on bat activity: towards improved clutter-based hypotheses," in *The Biology and Conservation of Australasian Bats*, ed. Law, B., Eby, P., Lunney, D. and Lumsden, D. (Sydney, NSW: Royal Zoological Society of New South Wales), 363–379.

Aldridge, H., and Rautenbach, I. (1987). Morphology, echolocation and resource partitioning in insectivorous bats. *J. Anim. Ecol.* 56:763. doi: 10.2307/4947

Ancillotto, L., Ariano, A., Nardone, V., Budinski, I., Rydell, J., and Russo, D. (2017). Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. *Agric. Ecosyst. Environ.* 241, 54–61. doi: 10.1016/j.agee.2017.03.001

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46. doi: 10.1111/j.1442-9993.2001.01070.pp.x

Barataud, M. (2020). *Acoustic Ecology of European Bats*. Paris: Museum national d'Historie naturelle.

Becker, R. A., Chambers, J. M., and Wilks, A. R. (1988). *The New S Language, a Programming Environment for Data Analysis and Graphics*. London: Wadsworth & Brooks/Cole Computer Science Series by Chapman & Hall.

Benton, T. G., Vickery, J. A., and Wilson, J. D. (2003). Farmland biodiversity: is habitat heterogeneity the key? *Trends Ecol. Evol.* 18, 182–188. doi: 10.1016/S0169-5347(03)00011-9

Blakey, R. V., Law, B. S., Kingsford, R. T., and Stoklosa, J. (2017). Terrestrial laser scanning reveals below-canopy bat trait relationships with forest structure. *Remote Sens. Environ.* 198, 40–51. doi: 10.1016/j.rse.2017.05.038

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008

Boughey, K. L., Lake, I. R., Haysom, K. A., and Dolman, P. M. (2011a). Effects of landscape-scale broadleaved woodland configuration and extent on roost location for six bat species across the UK. *Biol. Conserv.* 144, 2300–2310. doi: 10.1016/j.biocon.2011.06.008

Boughey, K. L., Lake, I. R., Haysom, K. A., and Dolman, P. M. (2011b). Improving the biodiversity benefits of hedgerows: how physical characteristics and the proximity of foraging habitat affect the use of linear features by bats. *Biol. Conserv.* 144, 1790–1798. doi: 10.1016/j.biocon.2011.02.017

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C., Nielsen, A., et al. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal*, 9:2, 378–400. doi: 10.32614/RJ-2017-066

Browning, E., Barlow, K. E., Burns, F., Hawkins, C., and Boughey, K. (2021). Drivers of European bat population change: a review reveals evidence gaps. *Mammal Rev.* 51, 353–368. doi: 10.1111/mam.12239

Burns, F., Eaton, M. A., Barlow, K. E., Beckmann, B. C., Brereton, T., Brooks, D. R., et al. (2016). Agricultural management and climatic change are the major drivers of biodiversity change in the UK. *PLoS One* 11:e0151595. doi: 10.1371/journal.pone.0151595

Butaye, J., Adriaens, D., and Honnay, O. (2005). Conservation and restoration of calcareous grasslands: a concise review of the effects of fragmentation and management on plant species. *BASE* 9

Cleveland, C. J., Betke, M., Federico, P., Frank, J. D., Hallam, T. G., Horn, J., et al. (2006). Economic value of the pest control service provided by Brazilian free-tailed bats in south-Central Texas. *Front. Ecol. Environ.* 4, 238–243. doi: 10.1890/1540-9295(2006)004[0238:evotpc]2.0.co;2

Davidai, N., Westbrook, J. K., Lessard, J.-P., Hallam, T. G., and McCracken, G. F. (2015). The importance of natural habitats to Brazilian free-tailed bats in intensive agricultural landscapes in the winter garden region of Texas, United States. *Biol. Conserv.* 190, 107–114. doi: 10.1016/j.biocon.2015.05.015

Davidson-Watts, I., Walls, S., and Jones, G. (2006). Differential habitat selection by *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. *Biol. Conserv.* 133, 118–127. doi: 10.1016/j.biocon.2006.05.027

Deák, B., Rádai, Z., Lukács, K., Kelemen, A., Kiss, R., Bátori, Z., et al. (2020). Fragmented dry grasslands preserve unique components of plant species and phylogenetic diversity in agricultural landscapes. *Biodivers. Conserv.* 29, 4091–4110. doi: 10.1007/s10531-020-02066-7

Dengler, J., Becker, T., Ruprecht, E., Szabó, A., Becker, U., Beldean, M., et al. (2012). *Festuco-Brometea* communities of the Transylvanian plateau (Romania)–a preliminary overview on syntaxonomy, ecology, and biodiversity. *Tuexenia* 32, 319–359.

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Guèze, M., Agard, J., et al. (2019). *Summary for policymakers of the global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*. Bonn: IPBES secretariat. doi: 10.5281/zenodo.3553579

Dietz, C., and Kiefer, A. (2016). *Bats of Britain and Europe*. London: Bloomsbury Publishing.

Dixon, M. D. (2012). Relationship between land cover and insectivorous bat activity in an urban landscape. *Urban Ecosyst.* 15, 683–695. doi: 10.1007/s11252-011-0219-y

Downs, N. C., and Racey, P. A. (2006). The use by bats of habitat features in mixed farmland in Scotland. *Acta Chiropterologica* 8, 169–185. doi: 10.3161/150811006777070893

Downs, N. C., and Sanderson, L. J. (2010). Do bats forage over cattle dung or over cattle? *Acta Chiropterologica* 12, 349–358. doi: 10.3161/150811010x537936

Duelli, P., and Obrist, M. K. (2003). Regional biodiversity in an agricultural landscape: the contribution of seminatural habitat islands. *Basic Appl. Ecol.* 4, 129–138. doi: 10.1078/1439-1791-00140

Entwistle, A., Racey, P., and Speakman, J. (1996). Habitat exploitation by a gleaning bat, *Plecotus auritus*. *Philos. Trans. R. Soc. Lond. B* 351, 921–931. doi: 10.1098/rstb.1996.0085

Ethier, K., and Fahrig, L. (2011). Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. *Landsc. Ecol.* 26, 865–876. doi: 10.1007/s10980-011-9614-2

Fenton, M. B. (1990). The foraging behaviour and ecology of animal-eating bats. *Can. J. Zool.* 68, 411–422. doi: 10.1139/z90-061

Fischer, J., Lindenmayer, D. B., and Manning, A. D. (2006). Biodiversity, ecosystem function, and resilience: ten guiding principles for commodity production landscapes. *Front. Ecol. Environ.* 4, 80–86. doi: 10.1890/1540-9295(2006)004[0080:BEFART]2.0.CO;

Frey-Ehrenbold, A., Bontadina, F., Arlettaz, R., and Obrist, M. K. (2013). Landscape connectivity, habitat structure and activity of bat guilds in farmland-dominated matrices. *J. Appl. Ecol.* 50, 252–261. doi: 10.1111/1365-2664.12034

Froidevaux, J. S., Laforge, A., Larrieu, L., Barbaro, L., Park, K., Fialas, P. C., et al. (2022). Tree size, microhabitat diversity and landscape structure determine the value of isolated trees for bats in farmland. *Biol. Conserv.* 267:109476. doi: 10.1016/j.biocon.2022.109476

Froidevaux, J. S. P., Zellweger, F., Bollmann, K., Jones, G., and Obrist, M. K. (2016). From field surveys to LiDAR: shining a light on how bats respond to forest structure. *Remote Sens. Environ.* 175, 242–250. doi: 10.1016/j.rse.2015.12.038

Fuentes-Montemayor, E., Goulson, D., Cavin, L., Wallace, J. M., and Park, K. J. (2013). Fragmented woodlands in agricultural landscapes: the influence of woodland character and landscape context on bats and their insect prey. *Agric. Ecosyst. Environ.* 172, 6–15. doi: 10.1016/j.agee.2013.03.019

Gehrt, S. D., and Chelsvig, J. E. (2004). Species-specific patterns of bat activity in an urban landscape. *Ecol. Appl.* 14, 625–635. doi: 10.1890/03-5013

Grüebler, M. U., Morand, M., and Naef-Daenzer, B. (2008). A predictive model of the density of airborne insects in agricultural environments. *Agric. Ecosyst. Environ.* 123, 75–80. doi: 10.1016/j.agee.2007.05.001

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., and Wiezik, M. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodivers. Conserv.* 22, 2131–2138. doi: 10.1007/s10531-013-0537-x

Hanspach, J., Fischer, J., Ikin, K., Stott, J., and Law, B. S. (2012). Using trait-based filtering as a predictive framework for conservation: a case study of bats on farms in southeastern Australia. *J. Appl. Ecol.* 49, 842–850. doi: 10.1111/j.1365-2664.2012.02159.x

Heim, O., Lenski, J., Schulze, J., Jung, K., Kramer-Schadt, S., Eccard, J. A., et al. (2018). The relevance of vegetation structures and small water bodies for bats foraging above farmland. *Basic Appl. Ecol.* 27, 9–19. doi: 10.1016/j.baae.2017.12.001

Heim, O., Lorenz, L., Kramer-Schadt, S., Jung, K., Voigt, C. C., and Eccard, J. A. (2017). Landscape and scale-dependent spatial niches of bats foraging above intensively used arable fields. *Ecol. Process.* 6:24. doi: 10.1186/s13717-017-0091-7

Heim, O., Schroder, A., Eccard, J., Jung, K., and Voigt, C. C. (2016). Seasonal activity patterns of European bats above intensively used farmland. *Agric. Ecosyst. Environ.* 233, 130–139. doi: 10.1016/j.agee.2016.09.002

Heim, O., Treitler, J. T., Tschapka, M., Knornschild, M., and Jung, K. (2015). The importance of landscape elements for bat activity and species richness in agricultural areas. *PLoS One* 10:e0134443. doi: 10.1371/journal.pone.0134443

Hodgson, J., Grime, J., Wilson, P., Thompson, K., and Band, S. (2005). The impacts of agricultural change (1963–2003) on the grassland flora of Central England: processes and prospects. *Basic Appl. Ecol.* 6, 107–118. doi: 10.1016/j.baae.2005.01.009

Jongman, R. H. (2002). Homogenisation and fragmentation of the European landscape: ecological consequences and solutions. *Landsc. Urban Plan.* 58, 211–221. doi: 10.1016/S0169-2046(01)00222-5

Jung, K., Kaiser, S., Bohm, S., Nieschulze, J., and Kalko, E. K. V. (2012). Moving in three dimensions: effects of structural complexity on occurrence and activity of insectivorous bats in managed forest stands. *J. Appl. Ecol.* 49, 523–531. doi: 10.1111/j.1365-2664.2012.02116.x

Jung, K., and Threlfall, C. G. (2018). Trait-dependent tolerance of bats to urbanization: a global meta-analysis. *Proc. R. Soc. B* 285:20181222. doi: 10.1098/rspb.2018.1222

Kalda, O., Kalda, R., and Liira, P. (2015a). Multi-scale ecology of insectivorous bats in agricultural landscapes. *Agric. Ecosyst. Environ.* 199, 105–113. doi: 10.1016/j.agee.2014.08.028

Kalda, R., Kalda, O., Lõhmus, K., and Liira, J. (2015b). Multi-scale ecology of woodland bat the role of species pool, landscape complexity and stand structure. *Biodivers. Conserv.* 24, 337–353. doi: 10.1007/s10531-014-0811-6

Kalka, M., and Kalko, E. K. (2006). Gleaning bats as underestimated predators of herbivorous insects: diet of *Micronycteris microtis* (*Phyllostomidae*) in Panama. *J. Trop. Ecol.* 22, 1–10. doi: 10.1017/S0266467405002920

Kalka, M. B., Smith, A. R., and Kalko, E. K. V. (2008). Bats limit arthropods and herbivory in a tropical Forest. *Science* 320:71. doi: 10.1126/science.1153352

Kelm, D. H., Lenski, J., Kelm, V., Toelch, U., and Dziock, F. (2014). Seasonal bat activity in relation to distance to hedgerows in an agricultural landscape in Central Europe and implications for wind energy development. *Acta Chiropterologica* 16, 65–73. doi: 10.3161/150811014x683273

Kusch, J., Weber, C., Idelberger, S., and Koob, T. (2004). Foraging habitat preferences of bats in relation to food supply and spatial vegetation structures in a western European low mountain range forest. *Folia Zool.* 53, 113–128.

Landesamt für Umwelt Brandenburg (2022). Lebensraumtypen [Online]. https://lfu.brandenburg.de/lfu/de/aufgaben/natur/biotopschutz/lebensraumtypen/# (Accessed May 4, 2022).

Leuschner, C., and Ellenberg, H. (2017). *Ecology of Central European Non-Forest Vegetation: Coastal to Alpine, Natural to Man-Made Habitats: Vegetation Ecology of Central Europe*. Berlin: Springer.

Lindborg, R., Plue, J., Andersson, K., and Cousins, S. A. (2014). Function of small habitat elements for enhancing plant diversity in different agricultural landscapes. *Biol. Conserv.* 169, 206–213. doi: 10.1016/j.biocon.2013.11.015

Loos, J., Krauss, J., Lyons, A., Föst, S., Ohlendorf, C., Racky, S., et al. (2021). Local and landscape responses of biodiversity in calcareous grasslands. *Biodivers. Conserv.* 30, 2415–2432. doi: 10.1007/s10531-021-02201-y

MacArthur, R. H., and MacArthur, J. W. (1961). On bird species diversity. *Ecology* 42, 594–598. doi: 10.2307/1932254

Martin, C. M., Arnett, E. B., Stevens, R. D., and Wallace, M. C. (2017). Reducing bat fatalities at wind facilities while improving the economic efficiency of operational mitigation. *J. Mammal.* 98, 378–385. doi: 10.1093/jmammal/gyx005

Matson, P. A., Parton, W. J., Power, A. G., and Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science* 277, 504–509. doi: 10.1126/science.277.5325.504

Meinig, H., Boye, P., Dähne, M., Hutterer, R., and Lang, J. (2020). Rote Liste und Gesamtartenliste der Säugetiere (Mammalia) Deutschlands. *Naturschutz und Biologische Vielfalt* 170:2. doi: 10.19213/972172

Mendes, E. S., Fonseca, C., Marques, S. F., Maia, D., and Pereira, M. J. R. (2017). Bat richness and activity in heterogeneous landscapes: guild-specific and scale-dependent? *Landsc. Ecol.* 32, 295–311. doi: 10.1007/s10980-016-0444-0

Miller, B. W. (2001). A method for determining relative activity of free flying bats using a new activity index for acoustic monitoring. *Acta Chiropterologica* 3, 93–105.

Monck-Whipp, L., Martin, A. E., Francis, C. M., and Fahrig, L. (2018). Farmland heterogeneity benefits bats in agricultural landscapes. *Agric. Ecosyst. Environ.* 253, 131–139. doi: 10.1016/j.agee.2017.11.001

Müller, J., Brandl, R., Buchner, J., Pretzsch, H., Seifert, S., Strätz, C., et al. (2013). From ground to above canopy—bat activity in mature forests is driven by vegetation density and height. *For. Ecol. Manag.* 306, 179–184. doi: 10.1016/j.foreco.2013.06.043

Müller, J., Mehr, M., Bässler, C., Fenton, M. B., Hothorn, T., Pretzsch, H., et al. (2012). Aggregative response in bats: prey abundance versus habitat. *Oecologia* 169, 673–684. doi: 10.1007/s00442-011-2247-y

Norberg, U. M., and Rayner, J. M. (1987). Ecological morphology and flight in bats (*Mammalia*; *Chiroptera*): wing adaptations, flight performance, foraging strategy and echolocation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 316, 335–427. doi: 10.1098/rstb.1987.0030

Obrist, M. K., Boesch, R., and Flückiger, P. F. (2004). Variability in echolocation call design of 26 Swiss bat species: consequences, limits and options for automated field identification with a synergetic pattern recognition approach. *Mammalia* 68, 307–322. doi: 10.1515/mamm.2004.030

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). "vegan: community ecology package". R package version 2.5-7 ed. Available at: https://CRAN.R-project.org/package=vegan (Accessed May 05, 2022).

Olimpi, E. M., and Philpott, S. M. (2018). Agroecological farming practices promote bats. *Agric. Ecosyst. Environ.* 265, 282–291. doi: 10.1016/j.agee.2018.06.008

Parsons, S., and Jones, G. (2000). Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. *J. Exp. Biol.* 203, 2641–2656. doi: 10.1242/jeb.203.17.2641

Pfalzer, G., and Kusch, J. (2003). Structure and variability of bat social calls: implications for specificity and individual recognition. *J. Zool.* 261, 21–33. doi: 10.1017/S0952836903003935

Poschlod, P., and Wallis de Vries, M. F. (2002). The historical and socioeconomic perspective of calcareous grasslands—lessons from the distant and recent past. *Biol. Conserv.* 104, 361–376. doi: 10.1016/S0006-3207(01)00201-4

Put, J. E., Fahrig, L., and Mitchell, G. W. (2019). Bats respond negatively to increases in the amount and homogenization of agricultural land cover. *Landsc. Ecol.* 34, 1889–1903. doi: 10.1007/s10980-019-00855-2

QGIS Development Team (2021). "QGIS geographic information system". Open source geospatial foundation project. Available at: http://qgis.osgeo.org (Accessed February 03, 2021).

R Core Team (2020). "R: A language and environment for statistical computing. R foundation for statistical computing". (Vienna, Austria). Available at: www.r-project.org (Accessed February 03, 2021).

Rasran, L., Diener, A., Pachinger, B., and Bernhardt, K.-G. (2018). Diversity of flower visiting insects in dry grasslands and vineyards close to the City of Vienna with special focus on wild bees. *Sociobiology* 65, 603–611. doi: 10.13102/sociobiology.v65i4.3370

Raven, P. H., and Wagner, D. L. (2021). Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proc. Natl. Acad. Sci.* 118:e2002548117. doi: 10.1073/pnas.2002548117

Rösch, V., Tscharntke, T., Scherber, C., and Batáry, P. (2015). Biodiversity conservation across taxa and landscapes requires many small as well as single large habitat fragments. *Oecologia* 179, 209–222. doi: 10.1007/s00442-015-3315-5

Rosenberg, D. K., Noon, B. R., and Meslow, E. C. (1997). Biological corridors: form, function, and efficacy. *Bioscience* 47, 677–687. doi: 10.2307/1313208

Russo, D., and Jones, G. (2002). Identification of twenty-two bat species (*Mammalia*: *Chiroptera*) from Italy by analysis of time-expanded recordings of echolocation calls. *J. Zool.* 258, 91–103. doi: 10.1017/S0952836902001231

Russo, D., and Voigt, C. C. (2016). The use of automated identification of bat echolocation calls in acoustic monitoring: a cautionary note for a sound analysis. *Ecol. Indic.* 66, 598–602. doi: 10.1016/j.ecolind.2016.02.036

Schloerke, B., Cook, D., Larmarange, J., Briatte, F., Marbach, M., Thoen, E., et al. (2021). 'GGally': Extension to 'ggplot2'. Available at: https://CRAN.R-project.org/package=GGally (Accessed April 24, 2022).

Schnitzler, H.-U., and Kalko, E. K. (2001). Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience* 51, 557–569. doi: 10.1641/0006-3568(2001)051[0557:EBIEB]2.0.CO;2

Schuldt, A., Ebeling, A., Kunz, M., Staab, M., Guimarães-Steinicke, C., Bachmann, D., et al. (2019). Multiple plant diversity components drive consumer communities across ecosystems. *Nat. Commun.* 10, 1–11. doi: 10.1038/s41467-019-09448-8

Skiba, R. (2009). *Europäische Fledermäuse* Magdeburg, Germany: NBB & VerlagsKG Wolf.

Söderström, B., Svensson, B., Vessby, K., and Glimskär, A. (2001). Plants, insects and birds in semi-natural pastures in relation to local habitat and landscape factors. *Biodivers. Conserv.* 10, 1839–1863. doi: 10.1023/A:1013153427422

Stahlschmidt, P., and Brühl, C. A. (2012). Bats at risk? Bat activity and insecticide residue analysis of food items in an apple orchard. *Environ. Toxicol. Chem.* 31, 1556–1563. doi: 10.1002/etc.1834

Stahlschmidt, P., Hahn, M., and Brühl, C. A. (2017). Nocturnal risks-high bat activity in the agricultural landscape indicates potential pesticide exposure. Frontiers in environmental. *Science* 5:62. doi: 10.3389/fenvs.2017.00062

Stahlschmidt, P., Pätzold, A., Ressl, L., Schulz, R., and Brühl, C. A. (2012). Constructed wetlands support bats in agricultural landscapes. *Basic Appl. Ecol.* 13, 196–203. doi: 10.1016/j.baae.2012.02.001

Starik, N., Göttert, T., Heitlinger, E., and Zeller, U. (2018). Bat Community responses to structural habitat complexity resulting from management practices within different land use types—a case study from north-eastern Germany. *Acta Chiropterologica* 20, 387–405. doi: 10.3161/15081109ACC2018.20.2.010

Statistical Office Berlin-Brandenburg (2020). *Statistisches Jahrbuch 2020*. Leck, Berlin.

Stoate, C., Boatman, N. D., Borralho, R., Carvalho, C. R., De Snoo, G., and Eden, P. (2001). Ecological impacts of arable intensification in Europe. *J. Environ. Manag.* 63, 337–365. doi: 10.1006/jema.2001.0473

Tilman, D., Fargione, J., Wolff, B., D'antonio, C., Dobson, A., Howarth, R., et al. (2001). Forecasting agriculturally driven global environmental change. *Science* 292, 281–284. doi: 10.1126/science.1057544

Toffoli, R. (2016). The importance of linear landscape elements for bats in a farmland area: the influence of height on activity. *J. Landsc. Ecol.* 9, 49–62. doi: 10.1515/jlecol-2016-0004

Treitler, J. T., Heim, O., Tschapka, M., and Jung, K. (2016). The effect of local land use and loss of forests on bats and nocturnal insects. *Ecol. Evol.* 6, 4289–4297. doi: 10.1002/ece3.2160

Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., and Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecol. Lett.* 8, 857–874. doi: 10.1111/j.1461-0248.2005.00782.x

Tscharntke, T., Steffan-Dewenter, I., Kruess, A., and Thies, C. (2002). Contribution of small habitat fragments to conservation of insect communities of grassland–cropland landscapes. *Ecol. Appl.* 12, 354–363. doi: 10.1890/1051-0761(2002)012[0354:COSHFT]2.0.CO;2

Verboom, B., and Huitema, H. (1997). The importance of linear landscape elements for the pipistrelle Pipistrellus *pipistrellus* and the serotine bat *Eptesicus serotinus*. *Landsc. Ecol.* 12, 117–125. doi: 10.1007/Bf02698211

Vrahnakis, M., Janišová, M., Rūsiņa, S., Török, P., Venn, S., and Dengler, J. (2013). "The European dry grassland group (EDGG): stewarding Europe's most diverse habitat type" in *Steppenlebensräume Europas*, ed. Baumbach, H, and Pfützenreuter, S. (Thüringen: Ministry for agriculture, Foerstry, Environment and Nature Protection), 417–434.

Wallis de Vries, M. F., and Van Swaay, C. A. (2009). "Grasslands as habitats for butterflies in Europe" in *Grasslands in Europe*, ed. Veen, P., Jedderson R., de Scmidt, J., and van der Straaten, J. (Leiden: KNNV publishing), 26–34. doi: 10.1163/9789004278103_004

Williams-Guillén, K., Perfecto, I., and Vandermeer, J. (2008). Bats limit insects in a neotropical agroforestry system. *Science* 320:70. doi: 10.1126/science.1152944

Wilson, J. B., Peet, R. K., Dengler, J., and Pärtel, M. (2012). Plant species richness: the world records. *J. Veg. Sci.* 23, 796–802. doi: 10.1111/j.1654-1103.2012.01400.x

Wood, E. M., Pidgeon, A. M., Radeloff, V. C., and Keuler, N. S. (2013). Image texture predicts avian density and species richness. *PLoS One* 8:e63211. doi: 10.1371/journal.pone.0063211

Young, H. S., McCauley, D. J., Galetti, M., and Dirzo, R. (2016). Patterns, causes, and consequences of anthropocene defaunation. *Annu. Rev. Ecol. Evol. Syst.* 47, 333–358. doi: 10.1146/annurev-ecolsys-112414-054142

Zeale, M. R., Davidson-Watts, I., and Jones, G. (2012). Home range use and habitat selection by barbastelle bats (*Barbastella barbastellus*): implications for conservation. *J. Mammal.* 93, 1110–1118. doi: 10.1644/11-MAMM-A-366.1

Zulka, K. P., Abensperg-Traun, M., Milasowszky, N., Bieringer, G., Gereben-Krenn, B.-A., Holzinger, W., et al. (2014). Species richness in dry grassland patches of eastern Austria: a multi-taxon study on the role of local, landscape and habitat quality variables. *Agric. Ecosyst. Environ.* 182, 25–36. doi: 10.1016/j.agee.2013.11.016

frontiers | Frontiers in Ecology and Evolution

TYPE Erratum
PUBLISHED 28 March 2023
DOI 10.3389/fevo.2023.1168225

Erratum: Structurally rich dry grasslands – Potential stepping stones for bats in open farmland

Frontiers Production Office*

Frontiers Media SA, Lausanne, Switzerland

KEYWORDS

dry grasslands, bats (*Chiroptera*), agricultural intensification, landscape, structural richness, acoustic monitoring, habitat fragments

An Erratum on
Structurally rich dry grasslands – Potential stepping stones for bats in open farmland

by Ewert, S. P., Knörnschild, M., Jung, K., and Frommolt, K.-H. (2023). *Front. Ecol. Evol.* 11:995133.
doi: 10.3389/fevo.2023.995133

Due to a production error, there was a mistake in Table 1 as published. There were several formatting issues. The corrected Table 1 appears below.

The publisher apologizes for this mistake. The original article has been updated.

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Frontiers Production Office
✉ production.office@frontiersin.org

SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 17 February 2023
ACCEPTED 17 February 2023
PUBLISHED 28 March 2023

CITATION
Frontiers Production Office (2023) Erratum: Structurally rich dry grasslands – Potential stepping stones for bats in open farmland. *Front. Ecol. Evol.* 11:1168225.
doi: 10.3389/fevo.2023.1168225

References

Boughey, K. L., Lake, I. R., Haysom, K. A., and Dolman, P. M. (2011b). Improving the biodiversity benefits of hedgerows: how physical characteristics and the proximity of foraging habitat affect the use of linear features by bats. *Biol. Conserv.* 144, 1790–1798. doi: 10.1016/j.biocon.2011.02.017

Downs, N. C., and Racey, P. A. (2006). The use by bats of habitat features in mixed farmland in Scotland. *Acta Chiropterologica* 8, 169–185. doi: 10.3161/150811006777070893

Frey-Ehrenbold, A., Bontadina, F., Arlettaz, R., and Obrist, M. K. (2013). Landscape connectivity, habitat structure and activity of bat guilds in farmland-dominated matrices. *J. Appl. Ecol.* 50, 252–261. doi: 10.1111/1365-2664.12034

Froidevaux, J. S. P., Zellweger, F., Bollmann, K., Jones, G., and Obrist, M. K. (2016). From field surveys to LiDAR: shining a light on how bats respond to forest structure. *Remote Sens. Environ.* 175, 242–250. doi: 10.1016/j.rse.2015.12.038

Fuentes-Montemayor, E., Goulson, D., Cavin, L., Wallace, J. M., and Park, K. J. (2013). Fragmented woodlands in agricultural landscapes: the influence of woodland character and landscape context on bats and their insect prey. *Agric. Ecosyst. Environ.* 172, 6–15. doi: 10.1016/j.agee.2013.03.019

Heim, O., Lenski, J., Schulze, J., Jung, K., Kramer-Schadt, S., Eccard, J. A., et al. (2018). The relevance of vegetation structures and small water bodies for bats foraging above farmland. *Basic Appl. Ecol.* 27, 9–19. doi: 10.1016/j.baae.2017.12.001

Heim, O., Treitler, J. T., Tschapka, M., Knornschild, M., and Jung, K. (2015). The importance of landscape elements for bat activity and species richness in agricultural areas. *PLoS One* 10:e0134443. doi: 10.1371/journal.pone.0134443

Kalda, O., Kalda, R., and Liira, P. (2015a). Multi-scale ecology of insectivorous bats in agricultural landscapes. *Agric. Ecosyst. Environ.* 199, 105–113. doi: 10.1016/j.agee.2014.08.028

Kalda, R., Kalda, O., Lõhmus, K., and Liira, J. (2015b). Multi-scale ecology of woodland bat the role of species pool, landscape complexity and stand structure. *Biodivers. Conserv.* 24, 337–353. doi: 10.1007/s10531-014-0811-6

Kelm, D. H., Lenski, J., Kelm, V., Toelch, U., and Dziock, F. (2014). Seasonal bat activity in relation to distance to hedgerows in an agricultural landscape in Central Europe and implications for wind energy development. *Acta Chiropterologica* 16, 65–73. doi: 10.3161/150811014x683273

Mendes, E. S., Fonseca, C., Marques, S. F., Maia, D., and Pereira, M. J. R. (2017). Bat richness and activity in heterogeneous landscapes: guild-specific and scale-dependent? *Landsc. Ecol.* 32, 295–311. doi: 10.1007/s10980-016-0444-0

Put, J. E., Fahrig, L., and Mitchell, G. W. (2019). Bats respond negatively to increases in the amount and homogenization of agricultural land cover. *Landsc. Ecol.* 34, 1889–1903. doi: 10.1007/s10980-019-00855-2

Stahlschmidt, P., Pätzold, A., Ressl, L., Schulz, R., and Brühl, C. A. (2012). Constructed wetlands support bats in agricultural landscapes. *Basic Appl. Ecol.* 13, 196–203. doi: 10.1016/j.baae.2012.02.001

Toffoli, R. (2016). The importance of linear landscape elements for bats in a farmland area: the influence of height on activity. *J. Landsc. Ecol.* 9, 49–62. doi: 10.1515/jlecol-2016-0004

Treitler, J. T., Heim, O., Tschapka, M., and Jung, K. (2016). The effect of local land use and loss of forests on bats and nocturnal insects. *Ecol. Evol.* 6, 4289–4297. doi: 10.1002/ece3.2160

Verboom, B., and Huitema, H. (1997). The importance of linear landscape elements for the pipistrelle Pipistrellus *pipistrellus* and the serotine bat *Eptesicus serotinus*. *Landsc. Ecol.* 12, 117–125. doi: 10.1007/Bf02 698211

TABLE 1 Final choice of local and landscape features and their abbreviations used for further analysis.

Local & Landscape features Measurements	Abbreviation	Relevance for bats found in:
Dry grassland ($r = 25$ m)		
Foliage height diversity (structural richness)	FHD	Froidevaux et al. (2016)
Landscape ($r = 200$ m)		
Forest: *Percentage cover*	For	Fuentes-Montemayor et al. (2013), Heim et al. (2015, 2018), Kalda et al. (2015b), Treitler et al. (2016)
Open land (pastures + cropland): *Percentage cover*	Open	Put et al. (2019)
Patches: *Counted number*	Patch	Mendes et al. (2017)
Urban area: **Distance*	DistUrb	Fuentes-Montemayor et al. (2013), Heim et al. (2015)
Water bodies: **Distance*	DistWat	Downs and Racey (2006), Stahlschmidt et al. (2012), Fuentes-Montemayor et al. (2013), Heim et al. (2015, 2018), Mendes et al. (2017)
Woody features: *Percentage cover* **Distance*	Wood DistWood	Verboom and Huitema (1997), Downs and Racey (2006), Boughey et al. (2011b), Frey-Ehrenbold et al. (2013), Kelm et al. (2014), Heim et al. (2015), Kalda et al. (2015a), Toffoli (2016)

Right column shows examples of literature where the landscape variable has shown a relevance for bats. *Distances were also measured when reaching outside of the 200 m radius.

TYPE Original Research
PUBLISHED 02 March 2023
DOI 10.3389/fevo.2023.1112596

EDITED BY
Grégory Mahy,
University of Liège,
Belgium

REVIEWED BY
Leonie K. Fischer,
University of Stuttgart,
Germany
Réka Kiss,
Hungarian Academy of Sciences,
Hungary

*CORRESPONDENCE
Mariana P. Fernandes
✉ mpfernandes@uevora.pt

SPECIALTY SECTION
This article was submitted to
Conservation and Restoration Ecology,
a section of the journal
Frontiers in Ecology and Evolution

RECEIVED 30 November 2022
ACCEPTED 31 January 2023
PUBLISHED 02 March 2023

CITATION
Fernandes MP, Matono P, Almeida E, Pinto-Cruz C and Belo ADF (2023) Sowing wildflower meadows in Mediterranean peri-urban green areas to promote grassland diversity.
Front. Ecol. Evol. 11:1112596.
doi: 10.3389/fevo.2023.1112596

Sowing wildflower meadows in Mediterranean peri-urban green areas to promote grassland diversity

Mariana P. Fernandes[1,2]*, Paula Matono[1], Erika Almeida[1], Carla Pinto-Cruz[3] and Anabela D. F. Belo[3]

[1]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Évora, Portugal, [2]cE3c – Center for Ecology, Evolution and Environmental Changes & CHANGE – Global Change and Sustainability Institute, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, Portugal, [3]MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Departamento de Biologia, Universidade de Évora, Pólo da Mitra, Évora, Portugal

Introduction: The increase of urban areas and their infrastructure network is homogenizing the landscape and threatening biodiversity and ecosystems functions and services. Wildflower meadows have a high biodiversity value and can prosper in degraded areas dominated by nitrophilous species, making them suitable to be used in peri-urban and urban areas to promote local flora, create habitat for pollinators and other small fauna, and increase overall biodiversity. Moreover, the application of wildflowers seed mixes suitable for rehabilitating anthropized environments should be restricted to native species of regional origin, and the results properly monitored. However, thorough monitoring of seed mixes evolution is uncommon. This study evaluates the effectiveness of a seed mix of wild native species developed to promote grassland diversity in Mediterranean peri-urban areas.

Methods: The study was divided into two sequential phases. Firstly, a preparatory phase consisted in developing two seed mixes and sowing them (autumn 2016) in *ex-situ* plots (three plots of 5×2m^2 per mix) at an experimental field to choose the one with the best performance. The second phase consisted of the *in-situ* application (autumn 2018) of the chosen seed mix by sowing 14 plots (10×2m^2) in pocket parks distributed along pedestrian trails of South Portugal. All plots were monitored through floristic surveys for two springs (*ex-situ* trials: 2017 and 2018; *in-situ* trials: 2019 and 2020).

Results: All sowed species germinated in the *in-situ* plots over the first 2 years. The seed mix application positively contributed to the floristic community, generating a significant increase in the total species richness, diversity, evenness, and vegetation cover. The seed mix establishment did not require watering nor soil fertilizing and the mowing frequency was low (once in late spring), contributing to sustainable and low-cost management of these green areas.

Discussion: The tested seed mix promoted native flora diversity rapidly and seems suitable for use in peri-urban context under identical climate conditions. Given the small number of native seed mixes tested in the Mediterranean, this study represents a contribution toward improved management standards of native flora diversity in Mediterranean green urban and peri-urban areas.

KEYWORDS

native flora conservation, native seed mixes, pedestrian path management, rehabilitation, semi-natural pastures

Introduction

Human activities have consistently affected natural and semi-natural habitats. Land use and land cover changes, particularly land taken by agriculture and urbanization, are transforming the terrestrial environment at unprecedented rates and scales, homogenizing the landscape and threatening biodiversity and ecosystems functions and services (Stoate et al., 2009; Seto et al., 2011). With the expansion of the urban network and the decline of natural ecosystems, urban areas management has a crucial role in preserving and enhancing biodiversity and presents unparalleled opportunities to enhance the urban systems' resilience and ecological functioning (Farinha-Marques et al., 2011; Elmqvist et al., 2015). Conservation of urban grasslands can reduce cities' ecological footprints and ecological debts while enhancing their inhabitants' resilience, health, and quality of life (Gómez-Baggethun and Barton, 2013). One way of diminishing the negative impact of cities is to use their green spaces to conserve biodiversity and improve these places' ecosystem value and function.

Natural and semi-natural grasslands are among the world's most threatened biomes (Janssen et al., 2016), making the conservation of their floristic assemblages, individual species, and ecological functions essential (Wesche et al., 2012). Urban areas usually have many patches of unused land (e.g., road and trail verges, roundabouts) and amenity areas (e.g., public parks, pocket parks, and lawns) frequently managed by regular mowing, whose potential for promoting biodiversity is often ignored (Blackmore and Goulson, 2014). These areas can be considered urban grasslands (Klaus, 2013), which are herbaceous, perennial landscapes of primarily turf-forming grass species adapted to semi-regular mowing and kept for aesthetic or recreational value (Thompson and Kao-Kniffin, 2017, 2019). Urban grasslands are considered novel habitats with a species assemblage partially altered due to an intense modification of biotic and abiotic conditions caused by the high level of human disturbance (Kowarik, 2011). Nevertheless, rehabilitated urban grasslands can maintain characteristic species of semi-natural grasslands and even rare species (Fischer et al., 2013a).

The urban green areas, including trail verges and pocket parks, are usually mown and, thus, kept in an early successional stage (Norton et al., 2019), promoting a vegetation composition and structure similar to natural and semi-natural grasslands. Due to their linear format, the trail verges can represent vegetation corridors between larger areas, such as parks or community gardens, promoting ecological connectivity. These linear infrastructures are, therefore, useful for grassland rehabilitation, i.e., for applying management actions that aim to reinstate a certain level of ecosystem functioning without the necessity to match up the biodiversity or integrity of the reference habitat with accuracy (Gann et al., 2019). Alter the management of these green areas to combine nature conservation with socio-economical aspects and landscape planning could be a win-win strategy for biodiversity conservation and improvement of socio-economic conditions (Gómez-Baggethun and Barton, 2013; Blackmore and Goulson, 2014; Bretzel et al., 2016).

Native wildflowers seed mixes sown in pocket parks and trail verges can enhance biodiversity and create an ecological continuity between urban and rural landscapes with low management costs (Blackmore and Goulson, 2014; Bretzel et al., 2016). Species-rich herbaceous communities, like grasslands, wildflower meadows and pastures, have a high biodiversity value and are adapted to soils with structural degradation and low organic matter content (Bretzel et al., 2016). Additionally, these floristic communities benefit from a regular mowing regime, which is the standard practice in urban green areas (Kelemen et al., 2014; Tälle et al., 2018). Thus, these herbaceous communities can constitute models to be adapted for landscape management of urban areas to increase biodiversity, create habitat for pollinators or small fauna, and conserve the local flora (Aldrich, 2002; Norton et al., 2019).

The effectiveness of wildflower meadows as a biodiversity promotion measure is related to the composition of the seed mix and its adaptation to local conditions, since this affects the number of species, flower abundance, plant species diversity, and vegetation structure in the sown areas (Haaland and Gyllin, 2011). Promoting a wide range of species in the urban grasslands increases life forms and survival strategies, favoring biodiversity promotion, and improving the establishment capacities of the floristic community (Bretzel et al., 2012). Although the species of semi-natural grasslands can adapt to urban environments, their choice should be judicious to guarantee an effective establishment: it is recommended to select native species, preferably of regional provenance, since they are well adapted to specific edaphoclimatic conditions, have fewer invasion risks, and help to conserve genetic diversity (Hufford and Mazer, 2003; Gann et al., 2019). The species selection should also consider the species' ability to survive in semi-arid and/or disturbed environments, the existence of an extended flowering period assured by the set of the species, the use of species with different life types (Bretzel et al., 2012), their germination behavior, particularly when stored seeds are used (Bhatt et al., 2019), and the species establishment features (Scheper et al., 2021). Using seed mixes containing annual, biennial, and perennial species is a holistic approach that makes it possible to combine the advantages of different life strategies. Annual species enhance the aesthetic value and may persist by self-seeding (Dunnett, 2011; Hoyle, 2016). In contrast, biennial and perennial species persist and flower over multiple years, reducing the need for re-sowing (Hoyle, 2016). The Mediterranean grassland communities naturally include patches of biennial and perennial species (Chust et al., 2006), and promoting these species in urban grasslands, along with annual species, increases the cover of a functional group already present in native ecosystems while maintaining the local biogeographic species pool (Filibeck et al., 2016). Furthermore, using species with different life strategies extends the flowering period: annual species flower in early spring and biennial and perennial species flower in late spring or summer (Bretzel et al., 2009, 2012). Promoting species with different flowering periods is beneficial because it increases the period of attractiveness to people and of resources available for pollinators (Hoyle et al., 2018).

In novel habitats, species assemblages can change the competitive dynamics between species (sown or spontaneous) compared to the habitats they usually inhabit (Hobbs et al., 2006). Consequently, the new floristic community will not have the same composition as in a non-anthropogenic environment (Fischer et al., 2013a). Therefore, species selection must consider the potential competition between all species (sown or spontaneous) One key question for the successful rehabilitation of urban grassland is how species cope with competitive pressure in an environment dominated by ruderal and exotic species (Fischer et al., 2013b), that are often competitive and tolerant to high stress and disturbance (Craine, 2005). The human disturbances in urban grasslands tend to favor the colonizing species with traits that increase a plant's ability to cope with stress and ruderal conditions (Fischer et al., 2013b). Usually, the competitive strategy of perennial species is less favored and increases the proportion of annual species (Fischer et al., 2013b).

Besides the ecological aspect, promoting semi-natural grasslands and wildflowers in urban areas also has economic, health promotion, and aesthetic value. To succeed, wildflowers should be sustainable, not require supplementary watering nor soil fertilizing, and have a low mowing frequency, and are, therefore, a low maintenance cost strategy (Bretzel et al., 2009; Köppler et al., 2014). Wildflowers can also provide ecosystem services like climate regulation, pollination, or soil and air quality improvement, being a link between urban environments and rural areas (Aldrich, 2002; Bretzel et al., 2016). This access to a wild natural environment positively impacts human health and quality of life and increments urban green spaces' aesthetic interest (Lindemann-Matthies and Brieger, 2016; Norton et al., 2019). The long-term rehabilitation of urban grasslands requires residents' acceptance and local authorities' willingness to maintain them (Norton et al., 2019). Recent studies (e.g., Southon et al., 2018; Norton et al., 2019) suggested that replacing some of the amenity grasslands with a range of different types of meadow vegetation is well received by the general population and can generate positive outcomes for both biodiversity and people. For instance, using seed mixes with an extended flowering period meets the aesthetic expectations of the population (Hoyle et al., 2017a; Colombo et al., 2021) and promotes pollinators (Hoyle et al., 2018; Angelella et al., 2019), favoring flora and fauna diversity and benefiting the human population. However, some aspects concerning the actual use of wildflowers in urban settings remain – the use of tall herbaceous and the vegetation's wild appearance close to residential areas is not always easily accepted (Hoyle et al., 2017b; Colombo et al., 2021). In the Mediterranean, the citizens' displeasure is even more significant after the flowering season when the vegetation dries up (Filibeck et al., 2016; Colombo et al., 2021). However, mowing the vegetation in late spring removes the dry biomass, minimizing the negative effect on the aesthetic appearance and reducing fire risk during the dry season. Informing the citizens about the benefits to biodiversity of rehabilitated urban grasslands and a wise selection of areas seems to be decisive for the population acceptance of these areas (Filibeck et al., 2016; Southon et al., 2018; Norton et al., 2019).

Using wildflowers or herbaceous seed mixes in urban environments is becoming widespread (Norton et al., 2019; Vega and Küffer, 2021); however, many seed mixes contain exotic species, use species not adapted to the specific edaphoclimatic conditions or do not consider the species' establishment and development capacity (Bretzel et al., 2016). In the Mediterranean region, native seeds' availability and applicability for urban environment rehabilitation is still limited (Bochet et al., 2010; Bretzel et al., 2012; de Vitis et al., 2017). Furthermore, this type of rehabilitation often reveals limited effectiveness, particularly in the Mediterranean region (Nunes et al., 2016), what highlights a still low predictive ability concerning rehabilitation trajectories and outcomes (Suding et al., 2015).

Applied research is needed, so that ecological rehabilitation of urban and peri-urban green areas, such as grasslands, can fulfil its potential and meet its goals as a conservation tool. We developed a seed mix of wild native species to promote floristic diversity in peri-urban areas and improve conditions for promoting small fauna (e.g., butterflies and small mammals). The seed mix was applied in pocket parks distributed throughout pedestrian trails of Southern Portugal. The study aims to (a) promote plant diversity, including endemic species, and favor habitat for small fauna, (b) evaluate the effect of a seed mix developed for Mediterranean areas on floristic diversity, evenness, and vegetation cover, and (c) evaluate the potential of peri-urban green areas as a tool for biodiversity conservation in the South of Portugal.

Materials and methods

Study area, species selection and seed collection

The study was carried out in Évora and Montemor-o-Novo (Central Alentejo – Southern Portugal) between 2016 and 2020 in a peri-urban context. The study area presents the typical land use pattern of the South of Portugal, with urban centers surrounded by cork oak forests (*montado*) and, nowadays, also by areas of intensive agriculture (e.g., vineyards and olive groves). Évora and Montemor-o-Novo are small cities with an urban area of 1,307 km^2 and 1,233 km^2 and a resident population of 53,577 and 15,799 inhabitants, respectively (FFMS, 2021). The area has a typical winter-wet and summer-dry pattern of the Mediterranean-type climate with a mean annual rainfall of 609 mm; the mean annual temperature is 15.9°C, and the warmest month is August (23.3°C) while the coldest is January (9.3°C; IPMA, 2019).

The study was divided into two subsequent phases: *ex-situ* (autumn 2016 to spring 2018) and *in-situ* trials (autumn 2018 to spring 2020; Figure 1). Firstly, *ex-situ* trials of two seed mixes were accomplished in an experimental field at Herdade da Mitra (University of Évora). Afterwards, *in-situ* application of the more appropriate seed mix was made in pocket parks distributed throughout pedestrian trails of Évora and Montemor-o-Novo.

Forty-one native species were selected to be used in the seed mixes, considering:

1. Their suitability to pedestrian trails and pocket parks, namely by being

- o resistant to drought and intense solar radiation;
- o annual plants that flower and set seed in early spring, ensuring their permanence in pedestrian trails and pocket parks (that are usually mown in late spring);
- o non-annual plants with subterranean organs that allow re-sprouting each year after being cut;
- o plants with no known toxicity.

2. Their conservation interest, namely by using endemic species.
3. The existence of well-established donor populations to collect seeds in the study area.

The species ecological characteristics were compiled from the literature (e.g., Castroviejo, 1986–2012; Flora-On, 2014; Flora Vascular, 2016) and benefited also from the authors' expert knowledge. The nomenclature of the species followed Flora Iberica (Castroviejo, 1986–2012). Seeds from the species selected were collected from plants growing in *montado* areas or in the intervened linear infrastructures surroundings during the springs and summers of

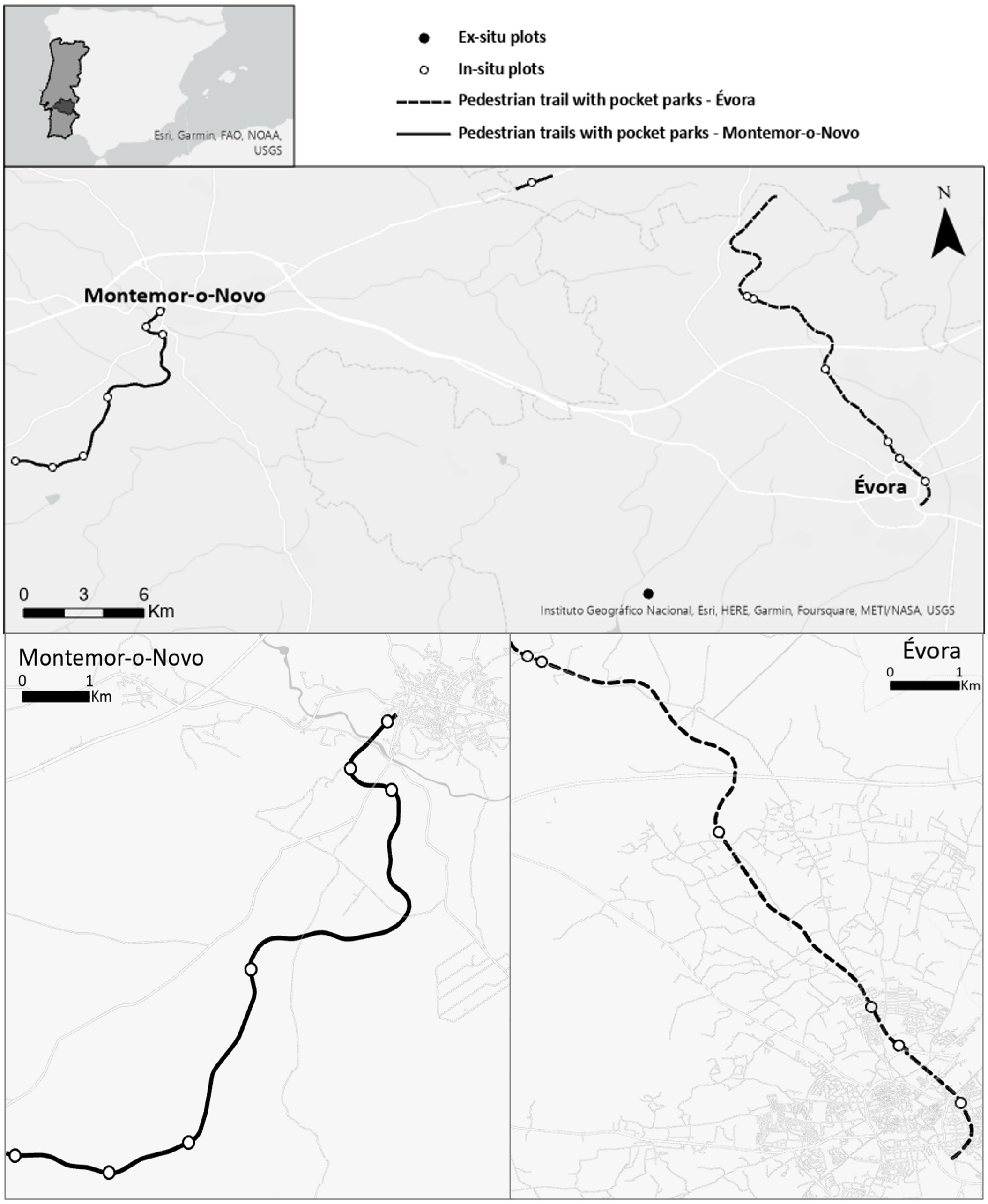


FIGURE 1
Ex-situ and *in-situ* plots installed on the experimental field of the University of Évora and pocket parks distributed throughout pedestrian trails of Évora (six plots) and Montemor-o-Novo (eight plots), respectively.

2016–2018, and their geographical coordinates were recorded with GPS technology (Garmin Montana 680 t). In all collecting campaigns, care was taken to never collect more than 20% of the seeds available at each site to avoid significant damage to the donor populations and assure natural regeneration (ENSCONET, 2009). In the seed bank of the University of Évora, the seeds were then cleaned, dehumidified at 15% RH (FITOCLIMA S600PDH – Aralab 1681; Portugal), stored in glass containers, and maintained in the dark at room temperature until being used in the *ex-situ* or the *in-situ* trials (autumn 2016 and 2018, respectively).

Viability trials

The selected species were submitted to viability trials in a germination chamber before each study phase to ensure that their viability was higher than 50% (Bellairs and Caswell, 2016), and only the seed lots – i.e., seeds belonging to the same batch of seeds, with a single reference number, origin, and history – meeting this criterion were considered suitable to be used in the trials. When using the same seed lot of a given species was not possible throughout the experiment (*ex-situ* and *in-situ* trials), viability trials were performed separately for each lot.

Seeds were germinated, without pre-germination treatments, in plastic Petri dishes (9 cm diameter) with agar (1%; VWR Chemicals) in a germination chamber (FITOCLIMA S600PLH – Aralab 1680; Portugal). For each species, four 25-seeds replicates were incubated with a photoperiod of 8 h of light and 16 h of darkness with an alternating temperature of 15/10°C. These incubation conditions simulate autumn conditions, which is the optimum germination season for most Mediterranean species, according to Copete et al. (2009) and Fernandes et al. (2021). Petri dishes were re-randomized daily to ensure that there were no systematic effects due to position within the germination chamber (Cerabolini et al., 2004).

The counting of germinated seeds was carried out daily over 30 days, and germinated seeds were removed from the Petri dishes. Though germination is considered to occur when the embryonic tissue emerges (Bewley et al., 2013), a more conservative approach was used by considering germination only when a radicle with at least half of the seed size emerged. Non-germinated seeds at the end of the trial were cut, and those with a well-developed, white, and firm embryo were considered viable (Gosling, 2003).

Ex-situ trials

The *ex-situ* trials occurred between November 2016 and June 2018 in an experimental field at Herdade da Mitra. Beside the experimental field there is a weather station managed by the University of Évora,[1] what allowed accurate recording of meteorological conditions. During the *ex-situ* trial, the maximum monthly rainfall was in March 2018 (207 mm). Rainfall was higher in the autumn/winter of 2017/2018 than in the remaining study periods. The mean monthly temperature ranged between 26.3 ± 4.3°C in August 2018 and 8.4 ± 2.1°C in January 2017 (ICT, 2021).

The 41 selected species were combined in two seed mixes with 23 species each (annex 1). Both seed mixes met the following criteria:

1. have a proportion of 30% Fabaceae, 30% Poaceae, 10% Asteraceae, and 30% other families. The chosen proportions intended to maximize floristic diversity by creating a mix with more than 15 species as recommended by Lepš et al. (2007) and Carter and Blair (2012), and to promote the existence of different life strategies (annual, biennial, and perennial species) as recommend by Bretzel et al. (2012) and Filibeck et al. (2016). Regular mowing of vegetation promotes grass dominance (Simões et al., 2014). As such, the balance between Poaceae, Fabaceae, and Asteraceae aimed to minimize grass dominance pace.
2. have at least one endemic species.
3. have at least one species for small fauna promotion, i.e., ensure the inclusion of at least one flora species that provides food or shelter for small animals, namely butterflies and small mammals.
4. provide a sequential and extended flowering period, offering aesthetic recreation for citizens and habitat and food for pollinators and small mammals.

Each seed mix was tested *ex-situ* in three plots (5 × 2 m^2 each) installed in November 2016 and one control plot (not sown) was also established. The plots had a slight slope (7°) and were exposed to full sun. The plots were previously harrowed and sowed with a density of 2,000 seeds/m^2. Seed density followed Hitchmough et al. (2008), according to which sowing with high densities (>1,200 seeds/m^2) positively contributes to promoting diverse floristic communities in grassland rehabilitation actions. The seeds did not receive any pre-germination treatment. The soil was then lightly raked to provide better contact between the seeds and the soil particles. No watering, fertilizing or pesticides were applied.

Floristic inventories in 50 × 50 cm^2 squares (three quadrats/plot evenly distributed throughout the center of the plot) were used to assess the total and species cover percentage (of both sown and spontaneous species) and the number of individuals of the sown species. Monitoring was done in 2017 and 2018 springs, to evaluate the mix performance over time. The plots were mowed at the end of the flowering season and after monitoring, to simulate the management regime usually carried out in marginal areas of pedestrian trails (June 2017 and 2018).

In-situ trials

The *in-situ* trial occurred between October 2018 and June 2020 in pocket parks distributed throughout pedestrian trails of Évora and Montemor-o-Novo. The pedestrian trails expand from the urban area to each city's surroundings. The pocket parks used in the study are in the marginal areas of the pedestrian trails and occur throughout the entire trail. The temperature and precipitation conditions of Évora and Montemor-o-Novo plots were considered separately, and the records were obtained from the nearest meteorological station. During the *in-situ* trial (Figure 2), rainfall was higher between November and December 2019 and the maximum monthly temperature was in August 2019 (ICT, 2021).

The selected seed mix underwent minor composition or proportion adjustments to improve the less positive aspects identified during *ex-situ* trials. In autumn 2018, the final seed mix (annex 2) was sown in 14 *in-situ* plots (10 × 2 m^2) in pocket parks distributed throughout pedestrian trails of Évora (six plots) and Montemor-o-Novo (eight plots). The sowing process was identical to that carried out in the *ex-situ* plots, with a density of 2,000 seeds/m^2 and the seeds used did not receive any pre-germination treatment. Twelve control plots (not sown) were also established in areas close to, and identical to, the sowing plots. All plots were in flat sites and exposed to full sun. No watering, fertilizing, or pesticides were applied. All the plots were mowed in late spring (June 2019 and 2020) as part of the current management regime of the pedestrian trails and respective pocket parks.

Citizens were informed of the ongoing conservation actions through signage placed at the beginning of pedestrian trails and in some of the intervened pocket parks. All the sown plots were marked with stakes.

The plots were monitored and sampled during the springs of 2017–2018 (before intervention: control and pre-sown plots) and 2019–2020 (after intervention: control and sown plots) through floristic inventories in 50 × 50 cm^2 squares (three quadrats/plot evenly distributed throughout the center of the plot). The following

---

[1] https://www.icterra.pt/g1/index.php/meteo-data/

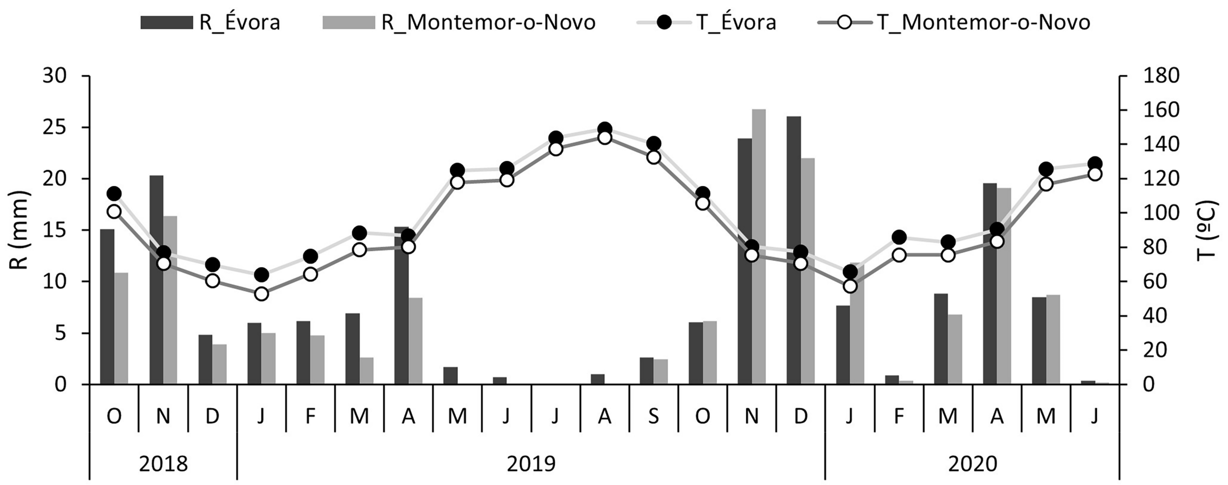


FIGURE 2
Total monthly rainfall (R) and mean monthly temperature (T) recorded accordingly the location of the *in-situ* plots (Évora or Montemor-o-Novo).

parameters were measured: total and species cover percentage (sown and spontaneous species), and the number of individuals of the sown species. The presence/absence of reproductive elements (flowers and/or fruits) of the sown species was also registered.

Data analysis

At the end of the viability trials and to ensure the seeds' quality, the viability percentage (VI) was calculated for each species lot considering the formula VI (%) = (V × 100)/NT, where V is the number of viable seeds (germinated seeds plus viable seeds that did not germinate) and NT is the total number of seeds.

In the *ex-situ* trials, the floristic community of the plots sown with the seed mixes was evaluated based on taxonomic terms and on the following parameters: sown species present in the floristic community (%), sown species that germinated (% and No.), global germination (%) of the seed mix (i.e., the total rate of sown seeds that germinated, regardless of species), cover of sown species (%), cover of spontaneous species (%), diversity of sown species (Shannon-Wienner index), and cover of sown species (evenness). The global germination of the mix was only evaluated in the first year after sowing (spring 2017).

The selection of the most appropriate mix of species to be applied in the *in-situ* plots was based on the best results obtained for these parameters. To support this process, the seed mixes under analysis, and respective control plot, were compared for each of these parameters using the Mann-Whitney non-parametric test.

For the purposes of comparing the results obtained in the *ex-situ* and *in-situ* trials, the initial floristic community of sites (control and pre-sown plots) was analyzed in terms of families, using the Wilcoxon non-parametric test. With the same purpose, this test was also used to compare the average annual values of air temperature (°C) and rainfall (mm) between the locations and years of the respective trials.

In the *in-situ* trials, both the seed mix's performance and the floristic community's evolution were evaluated. The global seed mix and species germination percentages were calculated only for the first spring after sowing (2019) and took into consideration a viability factor (number of non-viable seeds), ensuring a valid comparison between species and a correct results interpretation. The viability factor was applied for each species in a sown plot, considering the proportion of each species lot used in the experimental plot. The following formulas were applied:

1. Non-viable seeds (NS) = (NT-N) × (1-VI), where NT is the total number of seeds, N is the number of germinated seeds, and VI is the viability number obtained at the viability trial;
2. Germination percentage (%) = (N × 100)/(NT-NS), where N is the number of individuals (germinated seeds), NT is the number of total seeds, and NS is the number of non-viable seeds.

The germination percentage of the seed mix species was tested with the Mann-Whitney non-parametric test.

The floristic community of the plots was analyzed on taxonomic terms, as well as for sown richness (mean number of species), diversity (Shannon-Wiener Index), evenness (Pielou Index) and vegetation cover (%), considering sown species, spontaneous species (other native and ruderal) and the species total. Mann-Whitney non-parametric test was used to compare these parameters between years and between control and sown plots. To evaluate the efficiency of the seed mix, for each of the *in-situ* plots, the percentage increment of each of these metrics was calculated, according to the formula: Increase (%) = (final-initial)/|initial| × 100, where final corresponds to the value obtained in the plot after sowing and initial corresponds to the value obtained in the control plot/pre-sowing plot. The increment of each metric between control and sown plots was tested using the Mann-Whitney non-parametric test.

Data analysis was performed with the software Statistica 10, and the significance level was set at 0.05.

Results

The seeds' viability was high, with a mean percentage of 93 ± 9%. The viability percentages ranged between 57 and 100%. Sixty-eight percent of the lots registered a viability percentage above 95%, and only 7% registered a viability percentage lower than 65%. These results ensured the necessary seeds' quality for rehabilitation actions, such as the sowings carried out in this study.

The initial floristic community registered in the control and pre-sown plots of the *ex-situ* and *in-situ* trials did not show significant differences in the families' composition, allowing the comparability of the results, as well as the joint evaluation of post-sown data for the studied pedestrian trails. Also, the average air temperature and rainfall conditions did not reveal significant differences between the sites of *ex-situ* and *in-situ* trials, ensuring the comparability of the results without the need to consider the possible influence of these factors in the analysis of the results.

Ex-situ trials

In the *ex-situ* trials, both mixes registered significant differences and higher values in the parameters analyzed between control and sown plots ($p < 0.001$), therefore suggesting the effectiveness of the mixes, and supporting the restriction of the analysis to the direct comparison between the mixes under test to select the most suitable for the defined objectives. Mix 1 tended to have consistently higher values in most of the parameters analyzed (Table 1). However, despite this trend, for most parameters there were no significant differences between the two mixes in 2017 and 2018.

The floristic community of the plots sown with mix 1 registered a significantly higher percentage of the sown species and lower spontaneous species cover. In fact, although the percentage of sown species that germinated in 2017 was identical between the two mixes, in 2018 there was a significant decrease in the germination of the species in mix 2 compared to mix 1. Nevertheless, between years, the cover of sown species significantly increased for both mixes ($p < 0.05$).

The diversity and evenness of the germinated species were identical between the two seed mixes in 2017, and both registered a decreasing tendency regarding these parameters between 2017 and 2018.

In-situ trials

All the species of the final seed mix germinated in the *in-situ* plots in the first year, with global mean germination of 11% and maximum germination of 43% (*Briza maxima* L.). The germination percentage registered a high variability between species ($p < 0.001$). Seven species obtained germination values above 15% and, among them, *Briza maxima*, *Cynosurus echinatus* L., and *Silene gallica* L. obtained germination values above 30% (Figure 3).

There were no significant differences in the number of species and percentage of vegetation cover between the control and the pre-sown plots in the first monitoring (springs 2017 and 2018). The mean number of species increased in 2019 and 2020 ($p < 0.001$), following the germination of sown species, though other native and ruderal species also increased after the intervention (Figure 4). Therefore, both spontaneous and sown species increased the total of native flora species in both years ($p < 0.001$).

The introduction of the seed mix increased the vegetation cover of the sown plots ($p < 0.001$) and enhanced the existence of herbaceous vegetation layers, especially in 2020 (Figure 5). All the species of the seed mix persisted between 2019 and 2020, with a seed mix cover of 33% in both years. In 2019, the cover of spontaneous native species diminished in both control and sown plots. However, in 2020, the cover of ruderal species remained the same in the sown plots, and the cover of other spontaneous native species increased.

TABLE 1 Comparative analysis of the results obtained in 2017 and 2018 for different parameters of the floristic community (mean±standard error) in the two mixes sowed in the *ex-situ* plots at Herdade da Mitra.

Parameter	2017			2018		
	Mix 1	Mix 2	SD	Mix 1	Mix 2	SD
Sown species in the floristic community (%)	56.8 ± 3.2	43.5 ± 3.1	**	48.7 ± 3.0	38.6 ± 2.4	*
Sown species that germinated (%)	43.0 ± 1.5	38.2 ± 3.1		43.5 ± 4.2	29.5 ± 3.1	*
Sown species that germinated (no.)	9.9 ± 0.4	8.8 ± 0.7		10.0 ± 1.0	6.8 ± 0.7	*
Global germination of the seed mix (%)	8.3 ± 0.77	6.2 ± 0.9				
Cover of sown species (%)	48.8 ± 2.7	43.2 ± 4.6		68.6 ± 6.7	64.7 ± 8.7	
Cover of spontaneous species (%)	36.4 ± 3.8	62.8 ± 6.5	**	41.9 ± 8.2	47.0 ± 5.4	
Diversity of sown species (Shannon-Wienner index)	1.9 ± 0.06	1.9 ± 0.10		1.6 ± 0.09	1.1 ± 0.16	
Cover of sown species (evenness)	0.8 ± 0.02	0.9 ± 0.02		0.7 ± 0.03	0.6 ± 0.06	

The significant differences between each mix in each year are distinguished in column SD (significant differences): * ≤ 0.05; ** ≤ 0.01.

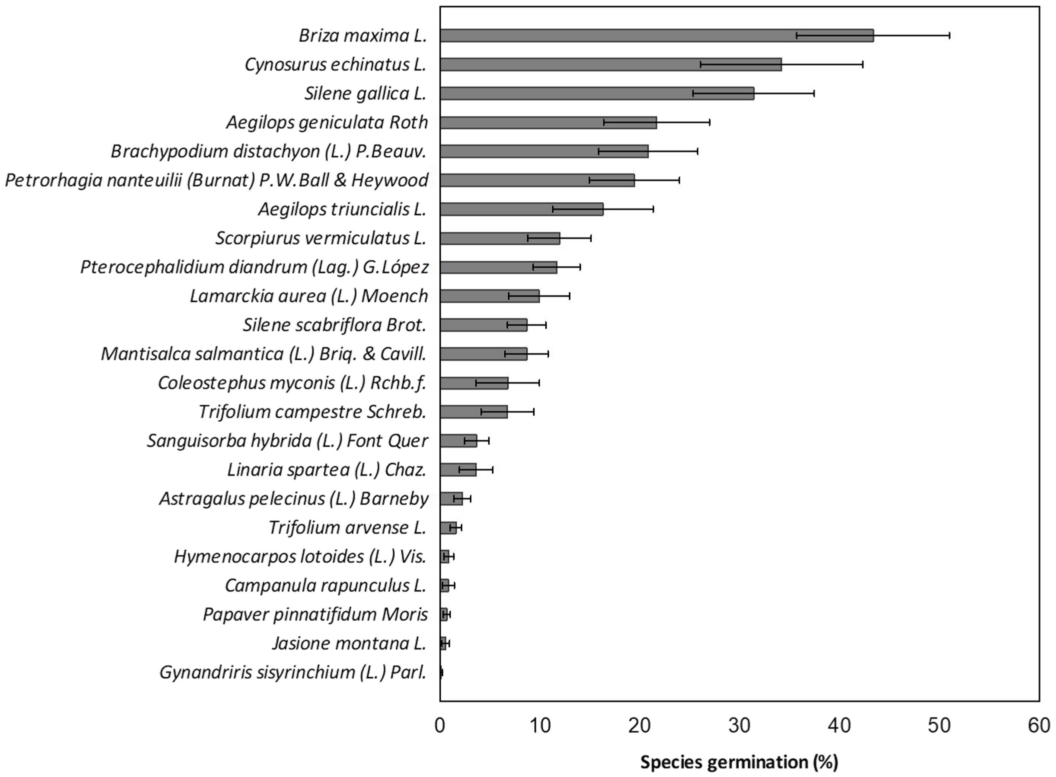


FIGURE 3
Germination percentage (mean±standard error) in 2019 of the seed mix species sown in the *in-situ* plots.

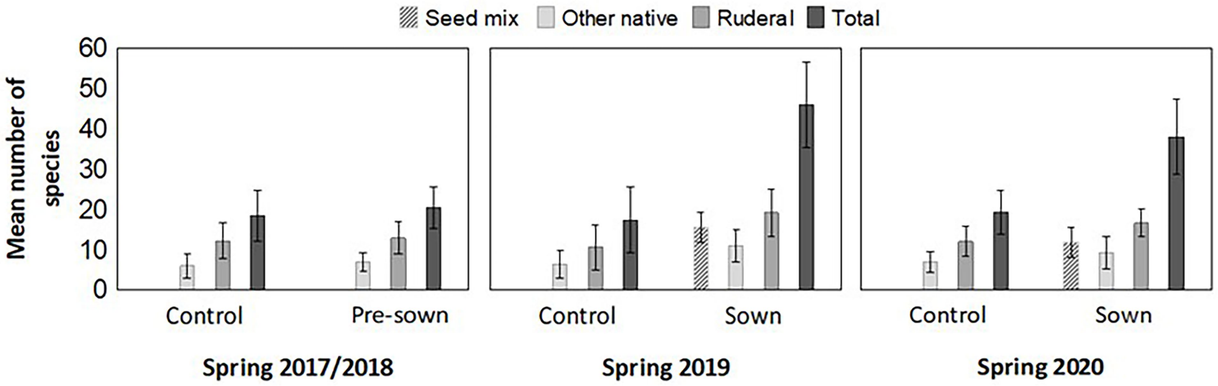


FIGURE 4
Number of species (mean±standard error) in control and *in-situ* sown plots, considering the seed mix species and spontaneous species (other native and ruderal species), and total richness.

Additionally, 96% of the sown species produced flowers and seeds in both monitoring years. The overall flowering period of the seed mix took place between March and June. The number of flora species that provide food or shelter for butterflies and small mammals increased in the sown plots through the germination and growth of species such as *Brachypodium distachyon* (L.) P.Beauv. and *Mantisalca salmantica* (L.) Briq&Cavill. The seed mix also contributed to the increase of endemic species in the sown plots through the germination and persistence of *Pterocephalidium diandrum* (Lag.) G.López, *Sanguisorba hybrida* (Link ex G.Don) Ces., and *Silene scabriflora* Brot.

Diversity and evenness were similar for control and pre-sown plots before the intervention (springs 2017 and 2018). The application of the final seed mix resulted in higher ($p < 0.001$) species diversity and evenness in the sown plots (Figure 6).

The application of the final seed mix generated a significant increase ($p < 0.001$) of the evaluated flora community parameters

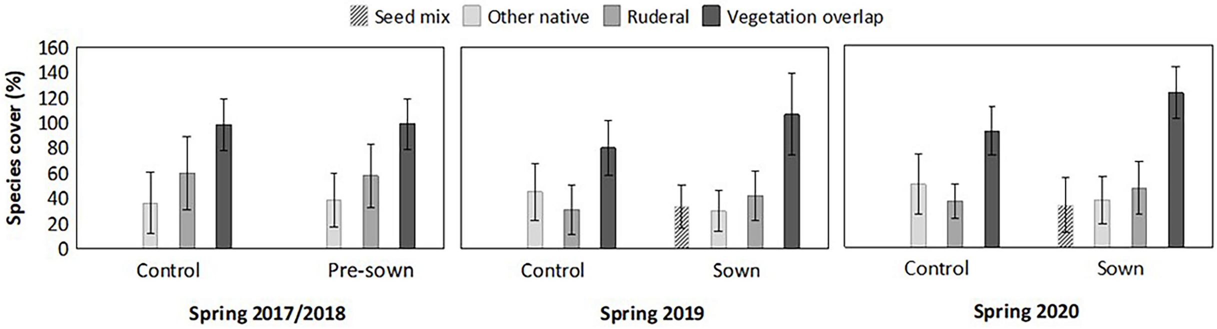


FIGURE 5
Species cover percentage (mean±standard error) in control and *in-situ* sown plots, considering the seed mix species and spontaneous species (other native and ruderal species), and vegetation overlap.

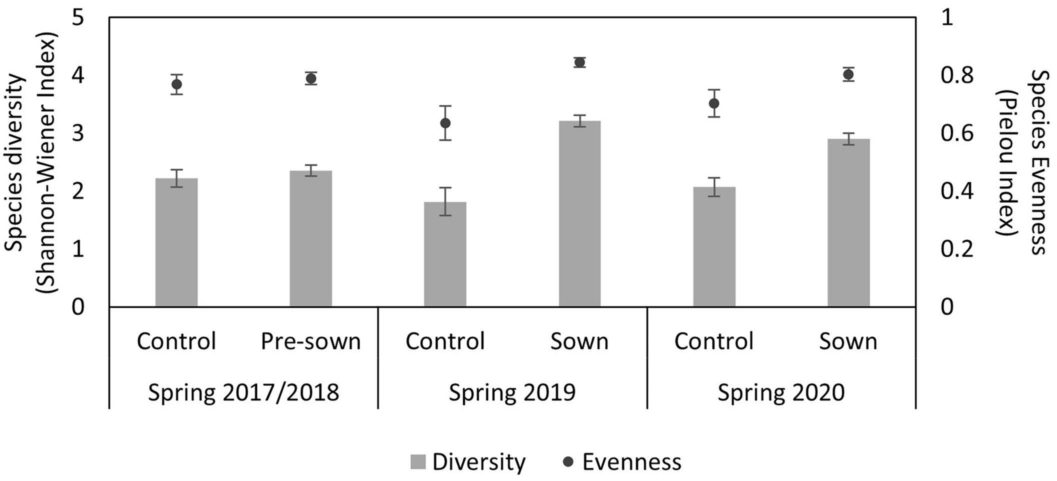


FIGURE 6
Species diversity (Shannon-Wiener index) and Evenness (Pielou Index) of the floristic community (mean±standard error) in control and *in-situ* sown plots.

(Table 2), highlighting the increase in total species richness (125%) and diversity (45%).

Discussion

In the *ex-situ* trials, the two mixes under analysis showed identical behavior for most parameters of the floristic community. This similarity between mixes can be explained by their composition, which includes some shared species or species with identical functional characteristics. In either mix, only some of the sown species managed to establish (mix 1: 43% in 2017 and 43.5% in 2018; mix 2: 38.2% in 2017 and 29% in 2018), which agrees with other studies carried out in semi-arid conditions (e.g., Tormo et al., 2006; Bochet et al., 2010), and the global germination percentage was less than 10%. This difference between the establishment performance of the sown species evidences the need to readjust the mixes' composition to ensure the success of the *in-situ* trials. Also, both mixes increased the vegetation cover in the second year despite a decrease in diversity and evenness of the sown species, reflecting a short-term persistence ability for some species and an increase in some species' dominance, namely grasses. The increase in the vegetation cover could be related to the precipitation variability between years, since the rainfall was higher in the autumn/winter of 2017/2018. The decrease in diversity and evenness of sown species is expected over time (e.g., Haaland and Gyllin, 2011; Angelella et al., 2019); however, in these seed mixes, it started shortly after the intervention (2nd spring), which may indicate the need to readjust the seed mix proportions. Despite the similarities between mixes, in the 2 years monitored, plots sown with mix 1 registered: (a) a higher percentage of sown species present in the floristic community, (b) a lower cover of spontaneous species, and (c) a steady percentage of sown species that germinated (about 43%) in both springs. The performance of mix 1 in these parameters may indicate better suitability to the edaphoclimatic conditions and increased capacity to fulfil the study aims of promoting native flora in the pedestrian trails' surrounding. Thus, mix 1 was considered most suitable for implementation in *in-situ* trials. Nevertheless, to ensure better performance, minor adjustments were made to the composition and proportion of mix 1 to incorporate the positive aspects of both mixes. These adjustments included (a) the redistribution of some

TABLE 2 Proportional increase of flora community parameters in the *in-situ* plots sown with the final seed mix.

Increase of flora community parameters	
Species richness	+125%
Species diversity (Shannon-Wienner index)	+45%
Species evenness (Pielou Index)	+14%
Vegetation cover	+17%

species proportion (e.g., *Medicago doliata* Carmign. was excluded from the mix, and the proportions of the remaining Fabaceae species were therefore slightly adjusted), (b) the addition of one species from the unselected mix (*Aegilops triuncialis* L.), and (c) the replacement of species by others from the unselected mix that obtained a better germination percentage (namely *Salvia verbenaca* L. and *Stipa capensis* Thunb. were replaced by *Petrorhagia nanteuilii* (Burnat) P.W.Ball&Heywood and *Lamarckia aurea* (L.) Moench, respectively) or have higher conservation interest (namely *Cerinthe major* L. and *Sanguisorba verrucosa* (Link ex G.Don) Ces. were replaced by *Pterocephalidium diandrum* and *Sanguisorba hybrida*, respectively).

In the *in-situ* plots, all sown species germinated in the first and second years after the intervention, reflecting a better performance of the final seed mix than the original seed mixes tested in the *ex-situ* trials and ensuring a more suitable selection of the species composition and proportion. The seed mix germination percentage in the *in-situ* trial was not high (11%) but follows what is usually registered in rehabilitation actions (Ceccon et al., 2016).

Applying seed mixes can be challenging, and even in places with low floristic diversity, such as many urban areas, sowing a wildflower mix does not automatically result in vegetation with the same composition as the seed mix (Bretzel et al., 2009; Fischer et al., 2013a; Schmidt et al., 2020). These differences in the species assemblage may occur due to the seed bank response or changes in the species' competitive dynamic (Hobbs et al., 2006; Fischer et al., 2013b). In this study, the application of the seed mix increased the species number in the *in-situ* plots due to the germination of sown species but also of spontaneous species (other native and ruderal species), most surely because these benefited from the disturbance of the soil seed bank produced at sowing (Hölzel et al., 2012). Therefore, this seed mix complemented and improved the floristic richness of the sown areas, as intended, without replacing the existing flora.

Developing a seed mix must consider the characteristics of the selected species and its applicability to the target place, considering factors such as soil type, soil seed bank, original land use, sowing method and weather conditions (Bretzel et al., 2012; Scheper et al., 2021). Despite considering other environmental factors (sun exposure and weather conditions), it was not possible to incorporate soil analyses that might help to explain the species' different behavior in *ex-situ* and *in-situ* trials. All tested seed mixes (*ex-situ* and *in-situ* trials) incorporated a sufficiently broad range of species to successfully establish the seed mix; although, testing the seed mix in different soil types would benefit the study by evaluating its adaptive ability.

The vegetation cover and the overlap of herbaceous species layers increased in the sown plots, ensuring a seed mix representation of 33% in the floristic community, and densifying the vegetation cover. The implemented seed mix is intended to promote grassland diversity and create favorable conditions for the permanency of small fauna such as butterflies and small mammals, what seems to be accomplished by increasing the number of these flora species, vegetation cover and vegetation layers. Among the sown species, the germination of *Brachypodium distachyon* and *Mantisalca salmantica*, for instance, stands out, because they contribute to the promotion of refuge and feeding areas for butterflies like *Melanargia ines* (Hoffmannsegg, 1804), *Thymelicus action* (Rottemburg, 1775), *Thymelicus lineola* (Ochsenheimer, 1808), and *Vanessa cardui* (Linnaeus, 1758) (Stefanescu, 1997; Maravalhas, 2003; Obregón and Prunier, 2014).

The final seed mix combined species from several families with different life forms (annual, biannual, and perennial), what increases the floristic community resiliency by allowing an extended flowering period and different survival strategies (Hoyle, 2016). Additionally, most species produced flowers and seeds, which is a good indicator of sustainability. The flowering period occurred throughout the spring (March to June), ensuring food availability for pollinators and an aesthetically pleasing appearance for pedestrians, an important feature in these circumstances, as noted by other studies (Lindemann-Matthies and Brieger, 2016; Hoyle et al., 2018; Scheper et al., 2021). An extended flowering period, without mowing, provides more opportunities for the self-reseeding of annual species (Hoyle, 2016), as the plants have more time to produce viable seeds, decreasing the need to reseed. The tested seed mix also allows a wide range of flower shapes and colors, contributing to more feeding opportunities for pollinators and improving the citizens' acceptance (Hoyle et al., 2018; Colombo et al., 2021; Scheper et al., 2021). Therefore, a seed mix composition that allows the longest and more diverse flowering season helps to increase biodiversity, minimizes reseeding costs, and improves the relationship between citizens and the urban rehabilitated grasslands. The long-term sustainability of urban rehabilitated grasslands depends on citizens' acceptance and local authorities' commitment (Norton et al., 2019); thus, ensuring a diverse and extended flowering season is a key factor when planning a seed mix composition.

The management timing and frequency of rehabilitated urban grasslands also play a decisive role in their long-term sustainability. Although mowing is essential to preserve grassland diversity (Tälle et al., 2018), recurrent mowing during the flowering season can make seed production unfeasible (e.g., Blažek and Lepš, 2015; Nakahama et al., 2016), decreasing the diversity of wildflower meadows over time. The studied pedestrian trails, and respective pocket parks, were managed by mowing the vegetation in late spring, allowing the sown species to complete their reproductive cycle, and contributing to a favorable evolution of the floristic community over the studied period. Based on these results and in other studies in the Mediterranean (Filibeck et al., 2016), we advise planning the mowing just after the flowering peak, i.e., between the middle and the end of June, in the case of the Mediterranean basin.

The three vegetation groups (other native, ruderal, and seed mix) under analysis were more balanced in the sown plots than in the control plots, with a decrease in the dominance of ruderal species and the fostering of less common spontaneous species. This balance between species follows other studies in urban areas (e.g., Fischer et al., 2013a,b; Norton et al., 2019), which verified a decrease in ruderal species in rehabilitated areas and the ability of meadow species to adapt to ruderal environments. The increment in the species diversity and evenness in the sown plots also corroborates the improvement of the flora community balance. Besides, the application of the seed mix increased the presence of species with conservation interest, like endemic species such as *Pterocephalidium diandrum*, *Sanguisorba hybrida*, and *Silene scabriflora*. As pointed out by Hobbs et al. (2006) and Fischer et al. (2013a), the coexistence of sown and spontaneous species results in a new floristic community that is probably more ruderal than natural or semi-natural grasslands. However, the establishment of less common species highlights the green urban areas' potential to preserve rare species of natural and semi-natural grasslands.

The application of the final seed mix in pocket parks distributed throughout pedestrian trails of South Portugal largely contributed to ameliorate the indicators of the floristic community, generating a quick and significant increase of total species richness in 125%, diversity in 45%, evenness in 14%, and cover in 17%. In addition, the establishment of this seed mix did not require watering nor soil fertilizing and the mowing frequency was low (once in late spring), following the requirements for implementing an ecologically sustainable and low-cost wildflower meadow (Bretzel et al., 2009; Köppler et al., 2014). The low requirements for maintaining rehabilitated urban grasslands are one of the significant advantages of their implementation from the point of view of policymakers and local authorities (Hoyle et al., 2017b). Thus, given these areas' economic and ecological advantages, ensuring that the population recognizes their ecological and social value can be a decisive factor in expanding their implementation.

The acceptance of native herbaceous vegetation in peri-urban or urban areas in the Mediterranean can be difficult, especially because of their appearance in summer (Filibeck et al., 2016; Colombo et al., 2021). Most native meadows and grassland species dry during summer, and the rehabilitated green areas can look unattractive and careless (Filibeck et al., 2016; Colombo et al., 2021). Mowing these areas in late spring can benefit the grassland species diversity, ensuring that they remain in an initial succession phase (Filibeck et al., 2016), and allowing a more acceptable aesthetic appearance during the dry season.

This study did not include acceptance population surveys; thus, we have no information about the population reaction in the intervened cities. To our knowledge, during the monitoring period, the sown plots were not vandalized, and the citizens did not react negatively to the urban grasslands' rehabilitation. Informational signs were placed at the beginning of pedestrian trails and some pocket parks, and the sown plots were marked with stakes. The signage of the rehabilitated areas allows citizens to be informed, what improves the ecological culture and increases the citizens' respect (Filibeck et al., 2016; Norton et al., 2019). Signaling the plots also allows, if necessary, to adapt the vegetation management and ensure that the rehabilitated areas are only mowed after the flowering season, contributing to long-term preservation (Norton et al., 2019). As referenced in other studies (e.g., Filibeck et al., 2016; Hoyle et al., 2017b; Southon et al., 2018; Norton et al., 2019), the size, location, distribution, and density of the biodiversity promotion plots in urbanized contexts can be critical factors in preserving the intervened areas and their acceptance by the citizens. Thus, the plots' small size, sparse distribution through pedestrian trails/pocket parks, and localization in peri-urban areas may have also contributed to their preservation. Furthermore, during the summer, the plots and the remaining vegetation were mowed, and the sown plots appearance was similar to the surrounding matrix.

Our study shows that the application of seed mixes with regional native species allows the promotion of native flora in Mediterranean peri-urban green areas, enhancing the potential of these spaces as tools for biodiversity conservation. Furthermore, the development and commercialization of native seed mixes could prevent the use of seed mixes imported from different biogeographical regions and, thus, diminish the risk of introducing exotic species with invasive ability.

The final seed mix seems suitable for replicating actions in a peri-urban context under identical climate conditions, namely in the south of the Iberian Peninsula. In the Mediterranean context and given the small number of native seed mixes tested, our study represents an innovation and provides practical insights into grassland rehabilitation, contributing to the improvement of plant diversity' management in peri-urban and urban areas. Nevertheless, further monitoring of the seed mix dynamics and evolution will be necessary since some changes may occur over the following years, and adjustments to species composition or proportions may be required. Additionally, the sustainability of the seed mix must be assessed in the long term, including an evaluation of the need for seeding reinforcement.

Data availability statement

The datasets presented in this article are not readily available because raw data were generated at the University of Évora, under the LIFE LINES project (LIFE14 NAT/PT/001081). Restrictions apply to the availability of these data, which were used under license for this study. Derived data supporting the findings of this study are available from the corresponding author MF on request. Requests to access the datasets should be directed to MF, mpfernandes@uevora.pt.

Author contributions

MF, CP-C, and AB conceived and designed the study. All authors participated in field work and contributed to data collection. PM analyzed data in collaboration with MF. MF, PM, and AB wrote and edited the manuscript, with inputs from the other authors. All authors contributed to the article and approved the submitted version.

Funding

This work is funded by National Funds through FCT – Foundation for Science and Technology under the Project UIDB/05183/2020.

Acknowledgments

MF was supported by a Ph.D. grant from FCT - Foundation for Science and Technology (2020.06339.BD). We thank the support of the project "LIFE LINES – Linear Infrastructure Networks with Ecological Solutions" (LIFE14 NAT/PT/001081), from the European Union LIFE Program. We also recognize the collaboration of Marlene Emídio during fieldwork and data collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1112596/full#supplementary-material

References

Aldrich, J. H. (2002). Factors and benefits in the establishment of modest-sized wildflower plantings: a review. *Native Plants J.* 3, 67–86. doi: 10.3368/npj.3.1.67

Angelella, G. M., Stange, L., Scoggins, H. L., and O'Rourke, M. E. (2019). Pollinator refuge establishment and conservation value: impacts of seedbed preparations, seed mixtures, and herbicides. *HortScience* 54, 445–451. doi: 10.21273/HORTSCI13600-18

Bellairs, S. M., and Caswell, M. J. (2016). Seed viability of native grasses is important when revegetating native wildlife habitat. *North Territ. Nat.* 27, 36–46.

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., and Nonogaki, H. (2013). *Seeds. 3rd Edn*. New York, NY: Springer New York.

Bhatt, A., Bhat, N. R., Suleiman, M. K., and Santo, A. (2019). Effects of storage, mucilage presence, photoperiod, thermoperiod and salinity on germination of *Farsetia aegyptia* Turra (Brassicaceae) seeds: implications for restoration and seed banks in Arabian Desert. *Plant Biosyst.* 153, 280–287. doi: 10.1080/11263504.2018.1473524

Blackmore, L. M., and Goulson, D. (2014). Evaluating the effectiveness of wildflower seed mixes for boosting floral diversity and bumblebee and hoverfly abundance in urban areas. *Insect Conserv. Divers* 7, 480–484. doi: 10.1111/icad.12071

Blažek, P., and Lepš, J. (2015). Victims of agricultural intensification: mowing date affects *Rhinanthus* spp. regeneration and fruit ripening. *Agric. Ecosyst. Environ.* 211, 10–16. doi: 10.1016/J.AGEE.2015.04.022

Bochet, E., Tormo, J., and García-Fayos, P. (2010). Native species for Roadslope revegetation: selection, validation, and cost effectiveness. *Restor. Ecol.* 18, 656–663. doi: 10.1111/j.1526-100X.2008.00496.x

Bretzel, F., Malorgio, F., Paoletti, L., and Pezzarossa, B. (2012). Response of sowed, flowering herbaceous communities suitable for anthropic Mediterranean areas under different mowing regimes. *Landsc. Urban Plan.* 107, 80–88. doi: 10.1016/j.landurbplan.2012.05.002

Bretzel, F., Pezzarossa, B., Benvenuti, S., Bravi, A., and Malorgio, F. (2009). Soil influence on the performance of 26 native herbaceous plants suitable for sustainable Mediterranean landscaping. *Acta Oecol.* 35, 657–663. doi: 10.1016/j.actao.2009.06.008

Bretzel, F., Vannucchi, F., Romano, D., Malorgio, F., Benvenuti, S., and Pezzarossa, B. (2016). Wildflowers: from conserving biodiversity to urban greening—a review. *Urban For. Urban Green.* 20, 428–436. doi: 10.1016/j.ufug.2016.10.008

Carter, D. L., and Blair, J. M. (2012). High richness and dense seeding enhance grassland restoration establishment but have little effect on drought response. *Ecological Applications* 22, 1308–1319. doi: 10.1890/11-1970.1

Castroviejo, S. (coord. gen.). (1986–2012). Flora Iberica 1–8, 10–15, 17–18, 21. CSIC, Madrid: Real Jardín Botánico Available at: http://www.floraiberica.es/miscelania/aviso_legal.php (Accessed January 22, 2019).

Ceccon, E., González, E. J., and Martorell, C. (2016). Is direct seeding a biologically viable strategy for restoring Forest ecosystems? Evidences from a meta-analysis. *Land Degrad. Dev.* 27, 511–520. doi: 10.1002/ldr.2421

Cerabolini, B., Andreis, R.De, Ceriani, R. M., Pierce, S., and Raimondi, B. (2004). Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens*. *Biol. Conserv.* 117, 351–356. doi: 10.1016/j.biocon.2003.12.011.

Chust, G., Pérez-Haase, A., Chave, J., and Pretus, J. L. (2006). Floristic patterns and plant traits of Mediterranean communities in fragmented habitats. *J. Biogeogr.* 33, 1235–1245. doi: 10.1111/j.1365-2699.2006.01492.x

Colombo, A. S., Berto, R., Ferrario, P., Toccolini, A., and Larcher, F. (2021). Wildflowers in urban design: an exploratory research of preference in Italian adults. *Vis. Sustain.* 2021, 35–52. doi: 10.13135/2384-8677/4599

Copete, M. Á., Herranz, J. M., and Ferrandis, P. (2009). Seed germination ecology of the endemic Iberian winter annuals *Iberis pectinata* and *Ziziphora aragonensis*. *Seed Sci. Res.* 19, 155–169. doi: 10.1017/S0960258509990079

Craine, J. M. (2005). Reconciling plant strategy theories of grime and Tilman. *J. Ecol.* 93, 1041–1052. doi: 10.1111/j.1365-2745.2005.01043.x

de Vitis, M., Abbandonato, H., Dixon, K. W., Laverack, G., Bonomi, C., and Pedrini, S. (2017). The European native seed industry: characterization and perspectives in grassland restoration. *Sustainability* 9, 1–14. doi: 10.3390/su9101682

Dunnett, N. (2011). Urban meadows: an ecological discussion. *Asp. Appl. Biol.*, 11–17.

Elmqvist, T., Setälä, H., Handel, S., van der Ploeg, S., Aronson, J., Blignaut, J., et al. (2015). Benefits of restoring ecosystem services in urban areas. *Curr. Opin. Environ. Sustain.* 14, 101–108. doi: 10.1016/J.COSUST.2015.05.001

ENSCONET (2009). ENSCONET - seed collecting manual for wild species. Available at: https://www.publicgardens.org/resources/ensconet-seed-collecting-manual-wild-species. January 10, 2023.

Farinha-Marques, P., Lameiras, J. M. M., Fernandes, C., Silva, S., and Guilherme, F. (2011). Urban biodiversity: a review of current concepts and contributions to multidisciplinary approaches. *Innovations* 24, 247–271. doi: 10.1080/13511610.2011.592062

Fernandes, M. P., Pinto-Cruz, C., Almeida, E., Emídio, M., Simões, M. P., Gazarini, L., et al. (2021). Seed germination of six Iberian endemic species – a contribution to enhance plant conservation. *Plant Biosyst.* 155, 1146–1152. doi: 10.1080/11263504.2020.1845841

FFMS (2021). Fundação Francisco Manuel dos Santos. Available at: https://www.pordata.pt/en/municipalities (Accessed January 3, 2023).

Filibeck, G., Petrella, P., and Cornelini, P. (2016). All ecosystems look messy, but some more so than others: a case-study on the management and acceptance of Mediterranean urban grasslands. *Urban For. Urban Green.* 15, 32–39. doi: 10.1016/j.ufug.2015.11.005

Fischer, L. K., von der Lippe, M., and Kowarik, I. (2013b). Urban grassland restoration: which plant traits make desired species successful colonizers? *Appl. Veg. Sci.* 16, 272–285. doi: 10.1111/j.1654-109X.2012.01216.x

Fischer, L. K., Von der Lippe, M., Rillig, M. C., and Kowarik, I. (2013a). Creating novel urban grasslands by reintroducing native species in wasteland vegetation. *Biol. Conserv.* 159, 119–126. doi: 10.1016/J.BIOCON.2012.11.028

Flora Vascular (2016). Flora Vascular – Toda la informacion detallada sobre la Flora Vascular. Available at: https://www.floravascular.com/ (Accessed December 15, 2021).

Flora-On (2014). Flora-On | Flora de Portugal interactiva. Sociedade Portuguesa de Botânica. Available at: https://flora-on.pt/ (Accessed December 15, 2021).

Gann, G. D., McDonald, T., Walder, B., Aronson, J., Nelson, C. R., Jonson, J., et al. (2019). International principles and standards for the practice of ecological restoration. Second edition. *Restor. Ecol.* 27, S1–S46. doi: 10.1111/rec.13035

Gómez-Baggethun, E., and Barton, D. N. (2013). Classifying and valuing ecosystem services for urban planning. *Ecol. Econ.* 86, 235–245. doi: 10.1016/j.ecolecon.2012.08.019

Gosling, P. G. (2003). "Viability testing" in *Seed Conservation: Turning Science Into Practice*. eds. R. D. Smith, J. B. Dickie, S. H. Linington, H. W. Pritchard and R. J. Probert (Kew, Great Britain: The Royal Botanic Gardens), 445–481.

Haaland, C., and Gyllin, M. (2011). "Sown wildflower strips – a strategy to enhance biodiversity and amenity in intensively used agricultural areas" in *The Importance of Biological Interactions in the Study of Biodiversity*. ed. J. López-Pujol (Barcelona: IntechOpen), 155–172.

Hitchmough, J., Paraskevopoulou, A., and Dunnett, N. (2008). Influence of grass suppression and sowing rate on the establishment and persistence of forb dominated urban meadows. *Urban Ecosyst.* 11, 33–44. doi: 10.1007/s11252-007-0041-8

Hobbs, R. J., Arico, S., Aronson, J., Baron, J. S., Bridgewater, P., Cramer, V. A., et al. (2006). Novel ecosystems: theoretical and management aspects of the new ecological world order. *Glob. Ecol. Biogeogr.* 15, 1–7. doi: 10.1111/J.1466-822X.2006.00212.X

Hölzel, N., Buisson, E., and Dutoit, T. (2012). Species introduction – a major topic in vegetation restoration. *Appl. Veg. Sci.* 15, 161–165. doi: 10.1111/j.1654-109X.2012.01189.x

Hoyle, H. (2016). Improving urban grassland for people and wildlife. Available at: http://www.relu.ac.uk/landbridge/LWEC%20PP%20Note%2032.pdf. (Accessed January 5, 2023).

Hoyle, H., Hitchmough, J., and Jorgensen, A. (2017a). All about the 'wow factor'? The relationships between aesthetics, restorative effect and perceived biodiversity in designed urban planting. *Landsc. Urban Plan.* 164, 109–123. doi: 10.1016/j.landurbplan.2017.03.011

Hoyle, H., Jorgensen, A., Warren, P., Dunnett, N., and Evans, K. (2017b). "Not in their front yard" the opportunities and challenges of introducing perennial urban meadows: a local authority stakeholder perspective. *Urban For. Urban Green.* 25, 139–149. doi: 10.1016/j.ufug.2017.05.009

Hoyle, H., Norton, B., Dunnett, N., Richards, J. P., Russell, J. M., and Warren, P. (2018). Plant species or flower colour diversity? Identifying the drivers of public and invertebrate response to designed annual meadows. *Landsc. Urban Plan.* 180, 103–113. doi: 10.1016/j.landurbplan.2018.08.017

Hufford, K. M., and Mazer, S. J. (2003). Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol. Evol.* 18, 147–155. doi: 10.1016/S0169-5347(03)00002-8

ICT (2021). Atmospheric sciences water and climate. Available at: http://www.i-c-t.pt/g1/ (Accessed December 15, 2021).

IPMA (2019). Instituto Português do Mar e da Atmosfera. Available at: https://www.ipma.pt/pt/oclima/normais.clima/1971-2000/ (Accessed May 1, 2022).

Janssen, J. A. M., Rodwell, J. S., Criado, M. G., Arts, G. H. P., Bijlsma, R. J., and Schaminee, J. H. J. (2016). European red list of habitats: Part 2. Terrestrial and Freshwater Habitats.

Kelemen, A., Török, P., Valkó, O., Deák, B., Miglécz, T., Tóth, K., et al. (2014). Sustaining recovered grasslands is not likely without proper management: vegetation changes after cessation of mowing. *Biodivers. Conserv.* 23, 741–751. doi: 10.1007/s10531-014-0631-8

Klaus, V. H. (2013). Urban grassland restoration: a neglected opportunity for biodiversity conservation. *Restor. Ecol.* 21, 665–669. doi: 10.1111/rec.12051

Köppler, M. R., Kowarik, I., Kühn, N., and von der Lippe, M. (2014). Enhancing wasteland vegetation by adding ornamentals: opportunities and constraints for establishing steppe and prairie species on urban demolition sites. *Landsc. Urban Plan.* 126, 1–9. doi: 10.1016/j.landurbplan.2014.03.001

Kowarik, I. (2011). Novel urban ecosystems, biodiversity, and conservation. *Environ. Pollut.* 159, 1974–1983. doi: 10.1016/j.envpol.2011.02.022

Lepš, J., Doležal, J., Bezemer, T. M., Brown, V. K., Hedlund, K., Igual, A. M., et al. (2007). Long-term effectiveness of sowing high and low diversity seed mixtures to enhance plant community development on ex-arable fields. *Appl. Veg. Sci.* 10:97. doi: 10.1658/1402-2001(2007)10[97:LEOSHA]2.0.CO;2

Lindemann-Matthies, P., and Brieger, H. (2016). Does urban gardening increase aesthetic quality of urban areas? A case study from Germany. *Urban For. Urban Green.* 17, 33–41. doi: 10.1016/j.ufug.2016.03.010

Maravalhas, E. (2003). *As borboletas de Portugal.* Portugal: Edição Ernestino Maravalhas.

Nakahama, N., Uchida, K., Ushimaru, A., and Isagi, Y. (2016). Timing of mowing influences genetic diversity and reproductive success in endangered semi-natural grassland plants. *Agric. Ecosyst. Environ.* 221, 20–27. doi: 10.1016/j.agee.2016.01.029

Norton, B. A., Bending, G. D., Clark, R., Corstanje, R., Dunnett, N., Evans, K. L., et al. (2019). Urban meadows as an alternative to short mown grassland: effects of composition and height on biodiversity. *Ecol. Appl.* 29:e01946-1115. doi: 10.1002/eap.1946

Nunes, A., Oliveira, G., Mexia, T., Valdecantos, A., Zucca, C., Costantini, E. A. C., et al. (2016). Ecological restoration across the Mediterranean Basin as viewed by practitioners. *Sci. Total Environ.* 566-567, 722–732. doi: 10.1016/j.scitotenv.2016.05.136

Obregón, R., and Prunier, F. (2014). Diversidad y ecología de una comunidad de Papilionoidea (Lepidoptera) en el arroyo Pedroches y su entorno: un paraje natural periurbano a conservar (Córdoba, España). *Rev. Gaditana Entomol.* 1, 183–201.

Scheper, J., Bukovinszky, T., Huigens, M. E., and Kleijn, D. (2021). Attractiveness of sown wildflower strips to flower-visiting insects depends on seed mixture and establishment success. *Basic Appl. Ecol.* 56, 401–415. doi: 10.1016/j.baae.2021.08.014

Schmidt, A., Kirmer, A., Kiehl, K., and Tischew, S. (2020). Seed mixture strongly affects species-richness and quality of perennial flower strips on fertile soil. *Basic Appl. Ecol.* 42, 62–72. doi: 10.1016/j.baae.2019.11.005

Seto, K. C., Fragkias, M., Güneralp, B., and Reilly, M. K. (2011). A meta-analysis of global urban land expansion. *PLoS One* 6:e23777. doi: 10.1371/journal.pone.0023777

Simões, M. P., Belo, A. F., Pinto-Cruz, C., and Pinheiro, A. C. (2014). Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Span. J. Agric. Res.* 12, 633–643. doi: 10.5424/sjar/2014123-5255

Southon, G. E., Jorgensen, A., Dunnett, N., Hoyle, H., and Evans, K. L. (2018). Perceived species-richness in urban green spaces: cues, accuracy and well-being impacts. *Landsc. Urban Plan.* 172, 1–10. doi: 10.1016/j.landurbplan.2017.12.002

Stefanescu, C. (1997). Migration patterns and feeding resources of the painted lady butterfly, *Cynthia cardui* (L.) (Lepidoptera, Nymphalidae) in the northeast of the Iberian peninsula. *Misc.·Zool.* 20, 31–48.

Stoate, C., Báldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., et al. (2009). Ecological impacts of early 21st century agricultural change in Europe – a review. *J. Environ. Manag.* 91, 22–46. doi: 10.1016/j.jenvman.2009.07.005

Suding, K., Higgs, E., Palmer, M., Callicott, J. B., Anderson, C. B., Baker, M., et al. (2015). Committing to ecological restoration. *Science* 348, 638–640. doi: 10.1126/science.aaa4216

Tälle, M., Deák, B., Poschlod, P., Valkó, O., Westerberg, L., and Milberg, P. (2018). Similar effects of different mowing frequencies on the conservation value of semi-natural grasslands in Europe. *Biodivers. Conserv.* 27, 2451–2475. doi: 10.1007/s10531-018-1562-6

Thompson, G. L., and Kao-Kniffin, J. (2017). Applying biodiversity and ecosystem function theory to turfgrass management. *Crop Sci.* 57, S-238–S-248. doi: 10.2135/cropsci2016.05.0433

Thompson, G. L., and Kao-Kniffin, J. (2019). Urban grassland management implications for soil C and N dynamics: a microbial perspective. *Front. Ecol. Evol.* 7:315. doi: 10.3389/FEVO.2019.00315/BIBTEX

Tormo, J., Bochet, E., and García-Fayos, P. (2006). Is seed availability enough to ensure colonization success? An experimental study in road embankments. *Ecol. Eng.* 26, 224–230. doi: 10.1016/j.ecoleng.2005.10.003

Vega, K. A., and Küffer, C. (2021). Promoting wildflower biodiversity in dense and green cities: the important role of small vegetation patches. *Urban For. Urban Green.* 62:127165. doi: 10.1016/j.ufug.2021.127165

Wesche, K., Krause, B., Culmsee, H., and Leuschner, C. (2012). Fifty years of change in central European grassland vegetation: large losses in species richness and animal-pollinated plants. *Biol. Conserv.* 150, 76–85. doi: 10.1016/j.biocon.2012.02.015

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 17 April 2023
DOI 10.3389/fevo.2023.1040931

EDITED BY
Péter Török,
University of Debrecen, Hungary

REVIEWED BY
Jana Růžičková,
ELKH-ELTE-MTM Integrative Ecology Research Group, Hungary
Béla Tóthmérész,
University of Debrecen, Hungary

*CORRESPONDENCE
Olivier Blight
✉ olivier.blight@imbe.fr

[†]These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Conservation and Restoration Ecology, a section of the journal Frontiers in Ecology and Evolution

RECEIVED 09 September 2022
ACCEPTED 15 March 2023
PUBLISHED 17 April 2023

CITATION
Blight O, Geslin B, Mottet L and Albert CH (2023) Potential of RFID telemetry for monitoring ground-dwelling beetle movements: A Mediterranean dry grassland study.
Front. Ecol. Evol. 11:1040931.
doi: 10.3389/fevo.2023.1040931

Potential of RFID telemetry for monitoring ground-dwelling beetle movements: A Mediterranean dry grassland study

Olivier Blight*[†], Benoît Geslin[†], Lola Mottet[†] and Cécile H. Albert[†]

Avignon University, Aix-Marseille University, CNRS, IRD, IMBE, Avignon, France

Better understanding insects' movements could help preserve and restore the insect communities that are key to the functioning of grasslands. Recent technological advances have led to spectacular achievements in movement ecology, making it possible to track the individual movements of a wide variety of organisms, including the smallest. However, monitoring systems such as RFID tags may negatively impact an organism's life history, with potential consequences on the reliability of data and conclusions. This study explored the potential of passive RFID tags to track the movements of three small ground-dwelling beetle species, a predator (*Poecilus sericeus*, Carabidae), a detritivore (*Asida sericea*, Tenebrionidae) and a granivore (*Acinopus picipes*, Carabidae), in a Mediterranean dry grassland degraded by years of cultivation. First, we tested whether carrying tags might impact individuals' behaviour, using a before-and-after design under laboratory conditions. Despite a trend toward shorter displacements, we found no significant short-term effect of the tags on individuals' movements. Second, we tracked a total of 25 tagged beetles in their natural environment every 4 h for 48 h. We highlight the principal limitation of using passive tags with small terrestrial beetles: the antenna has to pass over the tags to detect them, which restricts tracking to a few consecutive days after which the probability of locating an individual is low. However, the data obtained sheds light on the biological rhythms and daily movement capabilities of our target species: *A. sericea* is more mobile and *P. sericeus* less mobile than expected. Such knowledge could help predict the species' ability to recolonise degraded areas, enabling appropriate restoration actions to be designed based on landscape ecology principles.

KEYWORDS

movement ecology, conservation, grassland, insects, radio-tracking

1. Introduction

The global decline of grasslands is making conservation and restoration actions a priority (Buisson et al., 2022). Usually, it is plant species that are targetted in restoration actions, under the general assumption that insect populations will naturally follow the restoration of plant communities (Hilderbrand et al., 2005). However, this assumption neglects key processes that underpin the establishment and persistence of insect populations in sites to be restored, such as their ability to naturally reach these areas.

Ensuring that species can move between habitats or from preserved to degraded areas is vital for their maintenance and for ecosystem functioning and restoration (Massol et al., 2011; Poli et al., 2020). Considering species movements should therefore be a prerequisite when planning species conservation and habitat restoration (Katzner and Arlettaz, 2020). Currently, however, movement data are too often neglected when implementing conservation plans (Allen and Singh, 2016), reducing the effectiveness of traditional approaches such as protected areas given the spatial scale of species' movements (Thirgood et al., 2004). One of the challenges in species conservation is therefore to determine how, where, when and why species move.

The recent emergence of the science of movement that provides detailed spatio-temporal data is a significant advance in species conservation (Allen and Singh, 2016). Movement processes inform the foraging ecology and dispersal capacity of organisms (Ramos-Fernández et al., 2004). Research in movement ecology is generating knowledge of factors like the extent and use of geographical ranges, migratory pathways, phenology and activity period, the knowledge required for flexible conservation strategies in space and time (Le Gouar et al., 2015; Bérces and Růžičková, 2019). For example, to estimate the migratory range of stag beetles (*Lucanus cervus*) as a measure of connectivity among neighbouring populations for future conservation measures, Rink and Sinsch (2007) equipped individuals with 350 mg transmitters. They observed that about 1% of males are capable of maintaining gene flux among nest sites within a radius of about 3 km. Thus, isolated populations more than 3 km apart have an increased probability of local extinction.

Movement ecology has evolved thanks to recent technological advances such as radio frequency identification, hereafter RFID (e.g., Moreau et al., 2011), harmonic radar (e.g., Milanesio et al., 2016) or radio telemetry tags also called very high frequency (VHF) radio telemetry (e.g., Růžičková and Veselý, 2016). These technologies have been widely used to study the space use and movement behaviour of vertebrates (Holyoak et al., 2008). In comparison, insect movement ecology remains relatively neglected, despite the recent miniaturisation of tracking devices that has enabled accurate estimation of movements, even for small organisms like insects (Reynolds and Riley, 2002; Milanesio et al., 2016). Riecken and Raths (1996) were the first to use small radio-transmitters (0.6–0.7 g) to study the dispersal and habitat use of a terrestrial ground-dwelling insect, *Carabus coriaceus*, a large beetle about 40 mm long. Since then, a wide array of ground and flying arthropods has been tracked (Kissling et al., 2014), from tarantulas (e.g., Janowski-Bell and Horner, 1999) and beetles such as Carabidae (e.g., Negro et al., 2008) to hymenopterans (Henry et al., 2012) and dragonflies (Moskowitz and May, 2017).

When used on insects, tracking devices are commonly glued either directly on their external tegument (e.g., Batsleer et al., 2020) or to a line that is itself fixed onto the insects' back (e.g., Vinatier et al., 2010). However, this equipment may constrain insect behaviour and affect different aspects of an arthropod's life history (e.g., energy, movement, foraging, mating), potentially reducing the reliability of data and conclusions (Henry et al., 2012). Batsleer et al. (2020) recently called for more systematic documentation of potential effects of tags on arthropods' behaviour. Their review found that only 12% of the papers quantified the impacts of tags on insects. Most of the remaining papers claimed no effect, based on tag/body weight rules. The 5% rule—developed for flying or swimming vertebrates—considers that a tag/body weight ratio above 5% induces significant impacts (Kenward, 2001). Boiteau and Colpitts (2001) recommended that tags should weigh no more than 23–33% of the beetle's acceptable extra loading to limit impacts on the number and quality of flights. However, such general rules do not make much sense, as tag impacts are most likely dependent on study species, tag type, sex and environmental context (Jepsen et al., 2005). For example, predator and scavenger beetles of similar size and weight are unlikely to be affected in the same way by the tag. Unlike scavengers, predators often have to move quickly and sometimes over large areas to detect and catch their prey, which may make them more sensitive to additional weight.

Each of the available technologies has its own advantages and drawbacks for the study of arthropod movements (Kissling et al., 2014; Batsleer et al., 2020). Active radio transmitters allow individuals to be tracked over distances of more than 300 m for ground-dwelling insects (Negro et al., 2008); however, the problem is the weight of the transmitter, sometimes as heavy as a given arthropod. Passive tags (harmonic radar and RFID) have been applied to a broader range of arthropods, but the short detection distance complicates the monitoring of arthropods in the field. How much of a drawback this is depends, however, on the structure of the habitat; passive tags may be more appropriate in open habitats such as grasslands, where tagged individuals are easier to detect. For smaller arthropod species, where tag weight makes passive tags the only option, current challenges in monitoring movements include the limited detection distance and potential impacts on the animals' behaviour.

This study investigated the potential of passive RFID transmitters to track the movements of three small ground-dwelling beetle species typical of the Plaine de la Crau (Southern France) dry grassland: a predator (*Poecilus sericeus,* Fischer von Waldheim, 1824, Carabidae), a detritivore [*Asida sericea* (Olivier, 1795), Tenebrionidae] and a granivore [*Acinopus picipes* (Olivier, 1795), Carabidae]. We first explored how the tags impacted beetle behaviour, using a before-and-after design under laboratory conditions. Then we tracked tagged individuals in their natural environment, located in a protected nature reserve. Our findings are discussed in terms of their relevance to the conservation of beetle species in a protected dry grassland.

2. Materials and methods

2.1. Study site

La Crau is a plain dominated by dry grasslands located in the former delta of the Durance river (Southern France), to the east of the Rhône valley and the Camargue. This region is characterised by a Mediterranean climate, with low annual precipitation (400–500 mm per year), long hot summers and mild winters (mean annual temperature: 14° C). On average, the strong predominant wind ("mistral") blows from the North–West 334 days per year and the sun shines 3,000 h per year, generating very high levels of evapotranspiration (Devaux et al., 1983). In La Crau dry grasslands, about 50% of the soil surface is covered with stones transported by the Durance river 650,000 to 30,000 years ago. The substratum is an impermeable conglomerate bedrock, 40 cm deep, which limits the accessibility of groundwater for vegetation. Our field experiment took place in the centre of the sheepfold "Peau de Meau" (43.571525°N, 4.831853°E, elevation 10 m, 160 ha). The site is representative of the remaining 11,500 ha of dry grassland vegetation typical of the area, i.e., dominated by *Brachypodium retusum* P. Beauv. L., Poaceae and *Thymus vulgaris* L., Lamiaceae (Buisson and Dutoit, 2004; Römermann et al., 2005).

2.2. Study species

We focussed on three apterous beetle species: *Asida (polasida) sericea* (Olivier 1795), *Poecilus sericeus* (Fischer von Waldheim, 1824), *Acinopus (acinopus) picipes* (Olivier, 1795) (**Figure 1**). They are among the most abundant non-flying beetle species on the La Crau dry grassland, *A. sericea* and *P. sericeus* often jointly accounting for nearly 40% of the individuals caught (Blight et al., 2011). The three species are of similar size, with average body length from 11 to 14 mm (Jeannel, 1942; Soldati, 2006), but belong to three different feeding guilds. As a *Tenebrionidae*, *A. sericea* is a detritivore species. *Acinopus picipes* is an omnivorous-granivorous species with a large head and stout mandibles (Talarico et al., 2016), whereas *P. sericeus* is a predator with an elongated body and long mandibles (Jeannel, 1942). Their life cycles also differ: *P. sericeus* overwinters in adult stage, *A. picipes* overwinters in larval stages (Talarico et al., 2016) and *A. sericea's* imago can be found all year long (Soldati, 2006).

2.3. Monitoring devices

Based on preliminary tests with harmonic radar technology (RH, unpublished results), we decided to use radio frequency identification (RFID) to monitor the beetles' movement. The major issue with harmonic radar is individuals' movements being hindered by the long wire antennas getting caught in vegetation, as described in O'Neal et al. (2004). Despite a shorter detection range, RFID eliminates the problem of the protruding antenna and allows tagged individuals to be distinguished according to unique signals. We used miniature passive RFID tags (Mini HPT8 PIT Tag, Biomark Inc., Boise, ID, USA) 8 mm long by 1.4 mm wide, i.e., shorter and narrower than our study species. These tags weigh about 30 mg, which means that individuals equipped with glued diodes carried roughly 30% of their body mass. Tagging our study individuals added 26% extra weight to *A. picipes*, which weighs on average 0.12 g, 35% to *A. sericea*, which weighs on average 0.14 g, and 26% to *P. sericeus*, which weighs on average 0.12 g (**Table 1**).

Tags were glued to the insect's elytra with cyanoacrylate glue (Super glue 3 power flex mini trio, Loctite, Henkel, Germ). We tracked the tagged insects with a hand-held battery-powered transponder reading device (HPR Plus Reader, Biomark) equipped with a 40 cm diameter portable antenna (BP Plus Antenna, Biomark). The antenna had to pass over a tag to detect it. Detection height was also limited to a few centimetres (up to 30 cm, depending on the presence of stones under which beetles can hide).

2.4. Lab experiments

We first conducted an experiment to assess the impact of laboratory conditions and tags on the movements of the three species. A control group ($N = 6$ for *P. sericeus* and $N = 5$ for *A. picipes* and *A. sericea*) was tested for signs of familiarisation with an arena where they were repeatedly released. We hypothesised that their exploratory behaviour might be reduced by repeated release in the arena, through habituation to this new context. Individuals were released three times in the centre of a 42 cm × 27 cm plastic box (hereafter the arena), its bottom covered with white blotting paper to increase contrast with the dark insects. They were filmed for 4:30 min using a camera (alternatively 2 cameras: Nikon Coolpix P100, 10 megapixels and Nikon Coolpix P7700, 12 megapixel) attached to a copy stand. A second group of individuals ($N = 10$ for *A. sericea*, $N = 14$ for *P. sericeus* and $N = 7$ for *A. picipes*) was tested for the effect of the tags on behaviour and movements. Each individual was released three times in the arena and their behaviour recorded. The first time, the individual was "undisturbed"; the second time, a drop of glue was placed on it; the third time, the tag was glued onto its back. With or without tags, the glue was allowed to dry for 20 min before the individual's movements were filmed for 4:30 min using the device described above. We hypothesised that small tags glued to their elytra would not alter the movement capability of these ground-dwelling non-flying beetles.

Temperature in the experimental room was about 23°C. Filming was done in daylight and additional artificial light was used to improve image quality and avoid shading. Note that if an insect did not move for the first 2 min, we switched to the next individual.

All the individuals used in these experiments were hand-caught in the field and stored in boxes with soil and rocks retrieved from the field. Boxes were kept moist and oxygenated. Lab experiments were conducted in the days following their capture.

Data for each individual was treated in three steps. First, pictures (1920 × 1088 px) were extracted from the films (30 frames per sec.) at regular 5-s intervals with VLC© (scene filter on and recording ratio of 150), yielding 54 images per film. Second, the individual's spatial coordinates in the arena were calculated *via* automated image analysis, as in Mallard et al. (2013). Third, two variables related to the individual's activity in the arena were calculated: (1) average distance travelled in 5 s. time steps (pixels), and (2) total area explored, defined by number of pixels visited in an arena divided into 200 square pixels.

The average behaviours of individuals from the different species and treatments were compared *via* repeated measures ANOVA (hereafter *anova*), which allows for the same individuals to be measured on the same outcome variables under different conditions (three consecutive runs with or without equipment), using the function *anova_test* from the package *rstatix* (Kassambara, 2022). We accounted for multiple comparisons with Holm's adjustment, less conservative than the Bonferroni method (Holm, 1979). Non-normal variables were transformed using the function *bestNormalize* (Peterson, 2021).

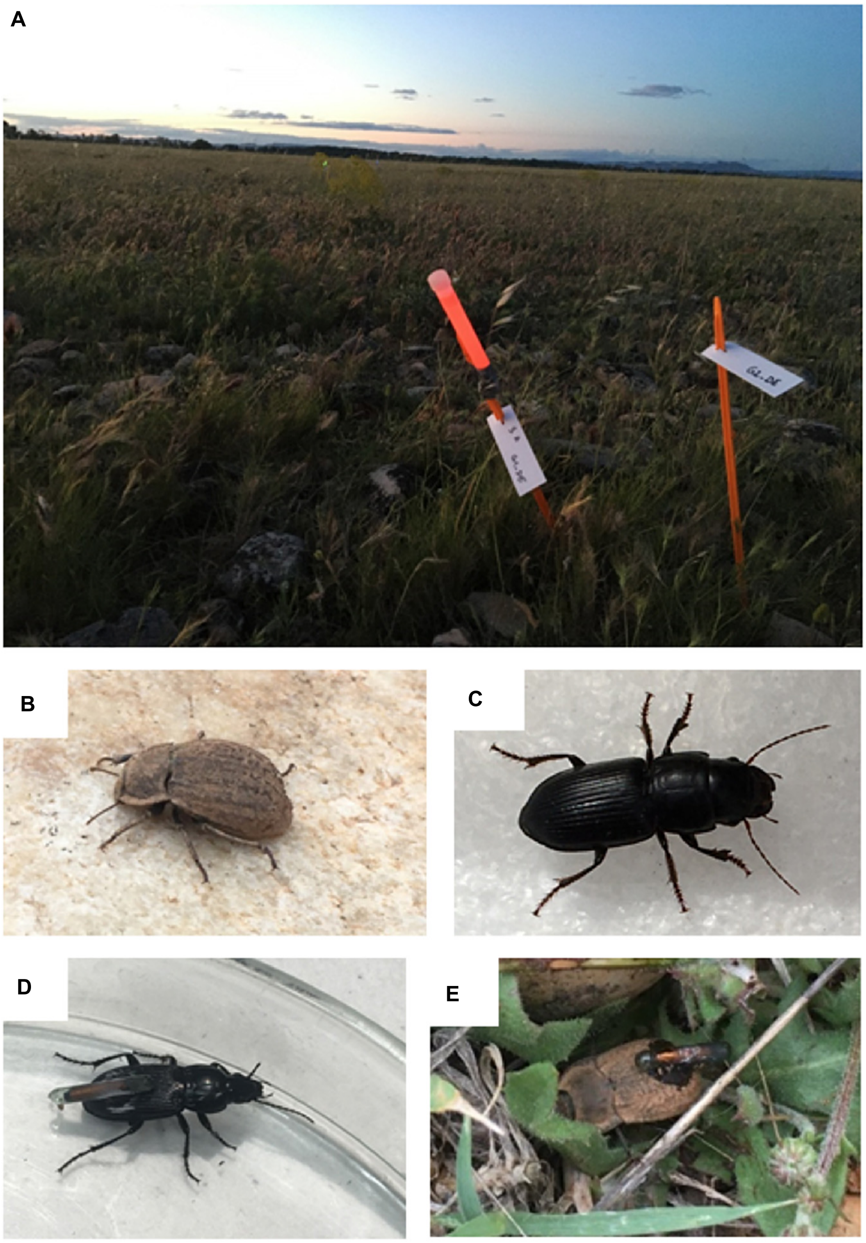


FIGURE 1
Study species and study area **(A)** the dry grasslands of la Crau and marking of release points, **(B)** *Asida sericea*, **(C)** *Acinopus picipes*, **(D)** *Poecilus sericeus* equipped with an RFID-tag on its back, **(E)** *Asida sericea* equipped with an RFID-tag and released in the field.

TABLE 1 Species weight alone (± SD) and with the diode, and additional weight generated.

Species	Weight alone (g)	Weight with diode (g)	Ratio	Add.weight (g)
A. picipes (*N* = 2)	0.120 ± 0.021	0.158 ± 0.011	1.248 ± 0.028	0.038
A. sericea (*N* = 8)	0.136 ± 0.032	0.180 ± 0.031	1.347 ± 0.107	0.044
P. sericeus (*N* = 14)	0.121 ± 0.016	0.152 ± 0.016	1.260 ± 0.043	0.030

To investigate how different individuals responded to our treatments, we used the intraclass correlation coefficient (ICC) with the function icc from package irr (Gamer et al., 2019). The ICC measures the reliability of ratings in inter-raters or in test–retest designs by comparing the variability of different ratings for the same individuals to the total variation across all ratings and all individuals (Shrout and Fleiss, 1979). Both agreement and consistency were measured. An ICC value above 0.9 means agreement (or consistency) is excellent, a value between 0.75 and 0.9 good, a value between 0.5 and 0.75 moderate and below 0.5 poor (Koo and Li, 2016). Non-normal variables were transformed using the function *bestNormalize* (Peterson, 2021).

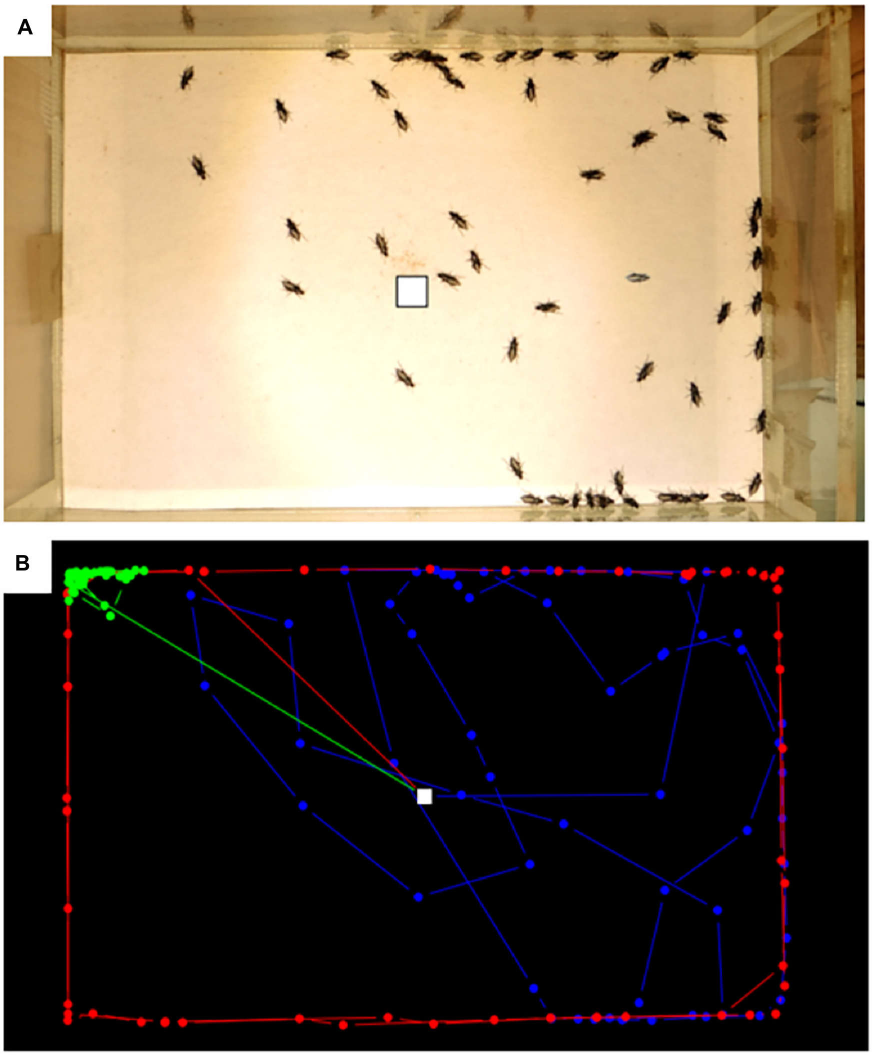


FIGURE 2
Monitoring beetle movements in the lab, **(A)** example of an experimental run with all-time steps overlapped, **(B)** example of three contrasting trajectories in blue, green and red. White square correspond to the release point.

2.5. Field experiment

The movements of individual beetles in the field were monitored every 4 h for 48 h (17–19 May 2021). Individuals were hand-caught in the morning of day 1 and kept in plastic boxes before their release. They were equipped with the same RFID tags as in the laboratory experiment (see above) and observed for 30 min minimum to confirm successful tag attachment. Individuals were released on day 1 at 14h30. Release points were located 10 m apart to facilitate detection procedures, reduce interference among tagged individuals and maximise the heterogeneity of stone and vegetation cover encountered in the dry grassland. Release points were located in a 40 m diameter circle to facilitate logistics and were marked with a labelled and coloured pole (Figure 1).

Initially, we released 25 individuals (N = 7 for *A. sericea* and N = 9 for *P. sericeus* and *A. picipes*), the number of individuals per species reflecting capture success. Every 4 h, all individuals were detected and their new position marked with a labelled pole. We recorded the time, the distance travelled since release with a decametre and the direction taken since release (deviation from compass direction, distance-bearing approach (Rùžièková and Elek, 2021) with the compass application of a smartphone (using a single iPhone 5S to avoid bias). This provided two daily time steps per time of day: afternoon (10:30–14:30, 14:30–18:30), night (18:30–22:30, 22:30–2:30), morning (2:30–6:30, 6:30–10:30).

When the individual appeared motionless after three consecutive detections, its physical condition was checked. Twelve hours after starting the experiment, there were two dead individuals and seven lost their tag. Nine additional individuals were released at 14h30 on day 2 (N = 3 for each species). One week later, an exhaustive field search was conducted over a circle of 6 m around the release points. The distance and angle to release point were recorded for each detected individual; undetected individuals were considered to have left these search areas.

This experiment took place under typical spring weather conditions, mainly sunny days with 30–50 km/h northerly wind and light rain ($<$ 0.5 mm for 2 h). The temperatures of the soil (10 cm below soil surface) and air (at 1.2 m above soil in the shade) were recorded with two loggers (Hobbo®, Pendant temp logger 64K). There was a clear night-day temperature contrast, with an average temperature of 18.8°C in the soil and 19.0°C in the air, a minimum temperature of 14.1°C in the soil and 8.6°C in the air and a maximum temperature of 27.0°C in the soil and 31.0°C in the air.

To test for differences between sessions in distance travelled for each species (morning, day and night), we used the non-parametric Kruskal-Wallis test from the package *rstatix* (Kassambara, 2022), as the data did not follow a normal distribution, even after transformation. Pairwise comparisons were performed using the pairwise.wilcox.test function with Holm's correction from the package *rstatix* (Kassambara, 2022).

All statistical analyses were performed using R Statistical Software (v4.1.2 R Core Team, 2022).

3. Results

3.1. Lab experiments

We observed strong individual variations in the behaviour of beetles in the arena, with some individuals circling along the edges, some widely exploring the surface and some remaining still in a corner (**Figure 2**). No clear sign of habituation to the arena was found, none of the analysis being significant (**Figure 3**). Moreover, the area explored by individuals differed in a completely idiosyncratic manner between arena sessions (ICC < 0.5) (**Figure 3B**). The distance travelled was moderately consistent (ICC ~ 0.6) for *A. sericea* and *A. picipes* across the three runs, with some individuals travelling fast while others remained slow.

In contrast, for the tag-effect test, we observed an overall effect on *A. sericea* [$F_{(2,18)} = 15.15$; $p < 0.001$]. Individuals covered significantly shorter distances per time step with the diode on their back than when carrying nothing (df = 2; $p = 0.004$) (**Figure 4A**), and also tended to cover shorter distances when they had glue alone on their backs compared to nothing (df = 2; $p = 0.067$) (**Figure 4B**). An overall effect was also observed on *A. picipes* [$F_{(2,12)} = 4.71$; $p = 0.031$] and *P. sericeus*, [$F_{(1.31,17.02)} = 5.22$; $p = 0.028$], but none of the comparisons were significant after applying Holm's correction. Neither carrying the glue alone nor the diode affected the area explored. None of the ICC values were above 0.5, indicating poor consistency in the individuals' behaviour and poor agreement in their ranking among treatments.

3.2. Field experiment

In the end, movement data was obtained on 25 individuals over 24 to 48 h: 8 *A. picipes* (out of 12 released), 10 *A. sericea* (out of 10 released) and 7 *P. sericeus* (out of 12 released). The other individuals were preyed on (2) or lost their diodes (7). One week later, within a 6 m radius of the release points, the field search identified 11 individuals that had moved farther than a week earlier (2 *A. picipes*, 5 *A. sericea*, 4 *P. sericeus*) and five that had not moved (due to territoriality, death or lost diode); the 9 other individuals were not found (**Table 2**, see **Appendix 1** for trajectory examples).

The distances travelled by the tagged individuals did not significantly differ between species due to high individual variability (all the metrics yielded the same results). After 24 h, *A. picipes* individuals were found approximately 0.81 metres from their starting point (max = 2.63 m), having travelled an average of 0.22 metre in 4 h (max = 2.17). *Asida sericea* individuals were found approximately 1.03 metres from their starting point (max = 3.62 m), having travelled an average of 0.33 metre in 4 h (max = 3.42). *Poecilus sericeus* individuals tended to travel less and were found approximately 0.5 metres from their starting point (max = 1.91 m), having travelled an average of 0.13 metre in 4 h (max = 1.98). On average, individuals started to move after 3.5-time steps for *A. picipes*, 4.6 for *A. sericea* and 5.8 for *P. sericeus*.

We observed a significant effect of time of day on movement patterns (df = 2; $\chi^2 = 12.78$; $p < 0.01$) (**Figure 5**). Individuals moved more at night than in the morning (df = 2; $p = 0.026$) and substantially more at night than in the afternoon (df = 2; $p = 0.003$), with no significant difference between morning and afternoon (df = 2; $p = 0.226$). The effect was, however, not equally strong for all three species. Time of day significantly affected the movements of *Asida sericea* (df = 2; $\chi^2 = 8.47$; $p = 0.015$), individuals moving significantly more at night than in the afternoon (df = 2; $p = 0.022$). In contrast, no significant effect was observed for *A. picipes* (df = 2; $\chi^2 = 4.6$; $p = 0.1$) or *P. sericeus* (df = 2; $\chi^2 = 1.27$; $p = 0.53$). Mobility was largely idiosyncratic, with some individuals moving more during the different time steps than others (**Figure 6**).

4. Discussion

Assessing the movement capability of insects is essential for the implementation of effective conservation and restoration measures. While the recent miniaturisation of tags is making it easier to track animals, including insects, there remain constraints such as the impact of tag weight on individual movement and the short detection distance. Here, we found weak effects of the tag on individual behaviours of three small ground-dwelling beetles in the laboratory, effects that were limited to the detritivore species. We obtained data on their daily behaviour by closely monitoring individuals in their habitat for 48 h and then a week later, but with a significant number of losses. In the light of these results, we discuss the implications of these beetle species' daily movements for the recovery of their populations in degraded areas of the Plaine de la Crau dry grassland.

4.1. Validation of the tracking technique

Previous radio tracking using passive tags was limited to beetles of more than 20 mm in length and a 0.4 g body mass (Kissling et al., 2014; Testud et al., 2019). Here, we demonstrated the validity of this technique even for smaller beetles (15 mm and 0.12 g). Despite a tendency toward shorter displacements, we observed few significant changes in the behaviour of tagged individuals under laboratory conditions. Only *A. sericea* covered significantly shorter distances per time step with the diode on. However, caution is required in interpreting these results; although we did not observe strong short-term behavioural effects, the tags could have impacts over several days or weeks.

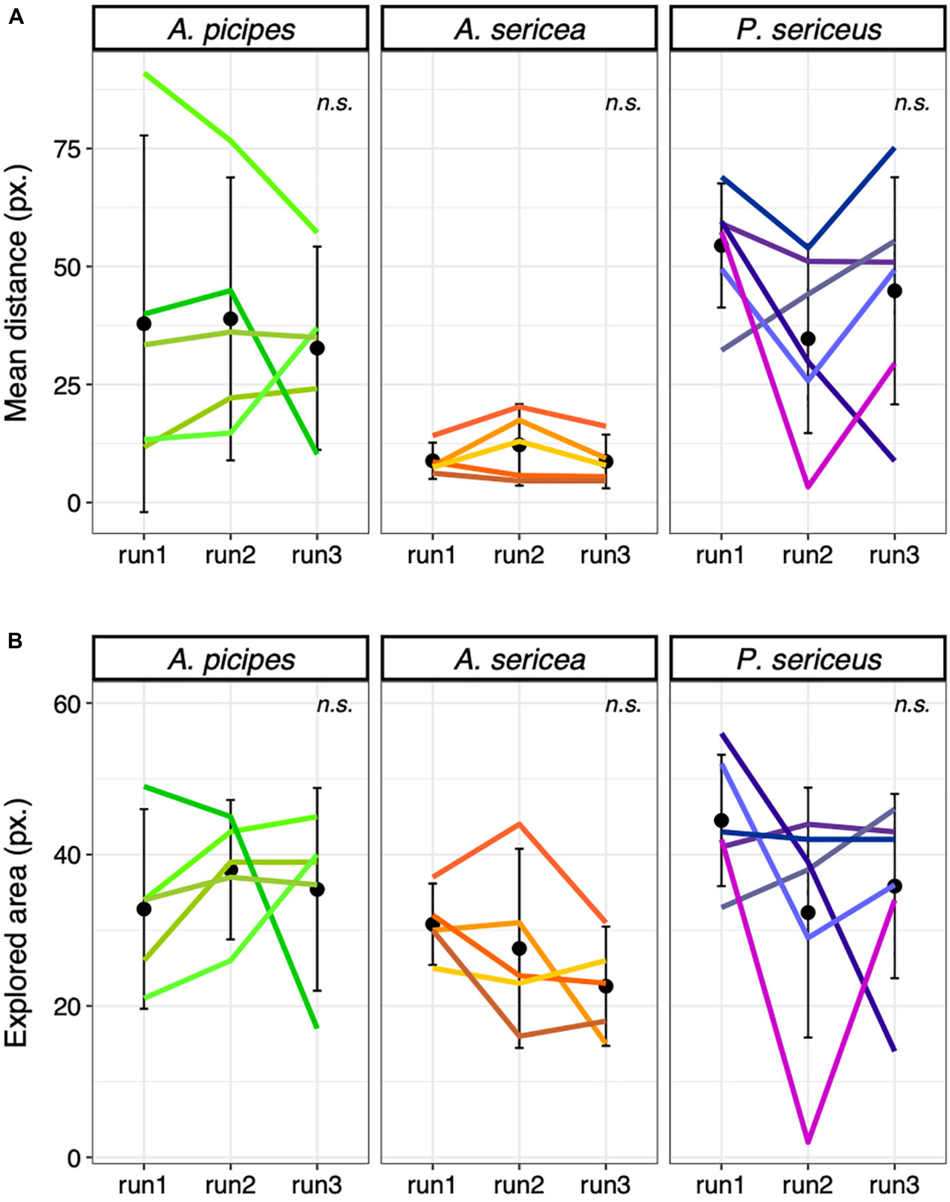


FIGURE 3
Control test, **(A)** Mean distance (± SE) travelled per time step among species and runs in the arena in pixels and **(B)** Mean explored area (± SE) among species and runs in the arena. n.s. = non-significant. Each coloured line represents an individual.

Because insects are known to be able to carry large extra loads for feeding or building activities, studies on insects' movements are generally conducted using a tag-to-body-mass ratio greater than the 5% body-mass rule established for vertebrates. Here, the full tagging system did not seem to strongly affect the beetles' movements, despite adding 26% to the body weight of *A. picipes*, 26% to *P. sericeus* and 35% to *A. sericea*. Actually, despite the growing use of telemetry techniques for tracking insects, their impacts are rarely assessed. Only 12% of the 173 studies reviewed by Batsleer et al. (2020) quantified the effect of tags on arthropods. In one example, the activity of the flightless cricket, *Gryllus locorojo*, reduced as tag weight increased from 18 to 127% of the individual's body mass (Kaláb et al., 2021). Boiteau et al. (2010), studying the beetle *Conotrachelus nenuphar*, reported that a tag weighing around 15% of its body mass did not reduce its mean walking speed. Another approach is to consider that tag weight must not exceed the maximum mass of an item the species can lift (Batsleer et al., 2020). This would, however, be difficult to determine for our studied species, which does not have to grab and carry food before eating it.

Our field experiment successfully used RFID to track small ground-dwelling beetle movements in the species' habitat. Despite the short detection distance (the antenna having to pass over the tag to detect it), we were able to locate all individuals except those that lost their tag or were preyed on. This meant we could track a reasonable proportion of the total tagged individuals (25 out of 34). However, our ability to locate individuals decreased greatly after 7 days, with only 16 out of 25 found after thoroughly searching an

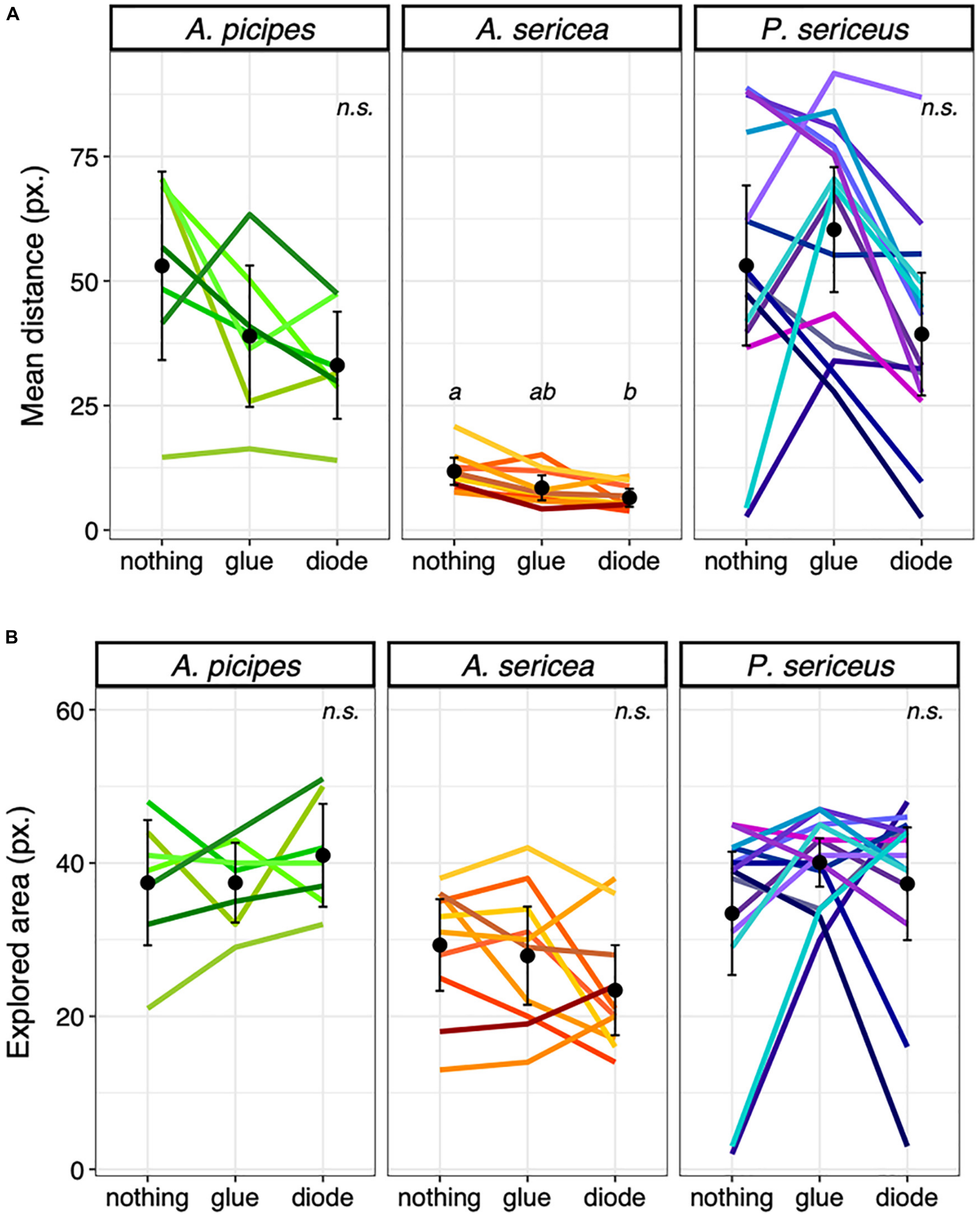


FIGURE 4
Effect of the diode, **(A)** Mean distance (± SE) travelled per time step among species and runs in the arena in pixels and **(B)** Mean explored area (± SE) among species and runs in the arena. Different letters indicate significant differences between runs ($P < 0.05$), n.s. = non-significant. Each coloured line represents an individual.

area 6 metres around the release point. It is therefore unlikely that we could track individuals over several weeks or months without close monitoring, which would be time-consuming and uncertain to succeed. The nature of the data collected is therefore limited to species' daily movements and foraging behaviour, without covering dispersal *sensu stricto* (Silcox et al., 2011). The method's applicability is also limited to species moving over short distances.

4.2. Species and individual-level data acquisition

Although limited in time, the data we collected provide useful knowledge on the behaviour and ecology of the three studied species, such as differences according to diet and daily activity. We observed differences between species in the distances travelled.

TABLE 2 Movements of the three species in the field.

Species	ID	Mean (m)	sd (m)	Max (m)	Sum (m)	1 week (m)
A. picipes	1	0.06	0.10	0.21	0.38	–
	2	0.09	23.00	0.78	1.03	0.86
	3	0.13	0.19	0.53	1.52	–
	4	0.22	0.22	0.58	2.60	1.92
	5	0.36	0.63	1.70	4.34	–
	6	0.53	0.73	2.18	6.37	–
	7	0.11	0.13	0.31	0.66	–
	8	0.02	0.05	0.13	0.13	–
A. sericea	1	0.84	0.83	3.01	10.13	-
	2	0.11	0.14	0.32	0.67	1.46
	3	0.10	0.19	0.59	1.24	–
	4	0.11	0.28	0.68	0.68	0.72
	5	0.18	0.20	1.05	2.12	3.95
	6	0.27	0.59	1.96	3.21	–
	7	0.01	0.04	0.13	0.13	5.47
	8	0.65	0.76	2.23	7.83	–
	9	0.65	1.05	3.43	7.80	5.45
	10	0.05	0.13	0.32	0.32	–
P. sericeus	1	0.22	0.25	0.49	1.34	–
	2	0.00	0.00	0.00	0.00	0.35
	3	0.04	0.10	0.31	0.51	2.33
	4	0.14	0.13	0.35	1.71	5.51
	5	0.16	0.31	0.80	1.91	–
	6	0.45	0.77	1.98	2.69	2.16
	7	0.02	0.05	0.13	0.13	–

Mean = mean distance (m) /time step; sd = sd distance (m) /time step; max = the maximum distance travelled (m) /time step; sum = the total distance travelled (m); 1 week = the distance (m) travelled from the release point after 1 week (the 11 individuals were all alive).

Unexpectedly, *Asida sericea* travelled the most and farthest in the field, whereas it was the least active and most impacted by the tag in the laboratory. *Poecilus sericeus*, the predator needing to search for prey, could have been expected to move around more than a detritivore or a granivorous species with easy access to food resources. Two non-exclusive explanations can be put forward. Firstly, while it appeared to be unaffected by the tag in the laboratory, *P. sericeus* may suffer more from the extra weight in a more structured environment of stones and dense vegetation, the species being thinner and lighter than the detritivore *A. sericea*. Secondly, *P. sericeus* might prefer to remain hidden from its prey, rather than actively hunting. Our experimental setup, limited in time and space, did not allow us to properly assess distance travelled and our arenas contained no hiding places.

Using both day and night sessions provided fundamental data on the phenology of the three species. They were all more active at night, which is relatively common in beetles, especially dark species. Dark nocturnal species have evolved as an interspecific adaptation to avoid predation (Hernández, 2002), and being nocturnal also allows them to avoid the warmer and drier daytime conditions.

Radio frequency identification tracking also provided useful data at the individual level. Confirming our observations in the laboratory, we recorded high within-species variability in behaviour. The individuals that were the least active the first day remained less active the following day, regardless of species. These consistent inter-individual differences might suggest the presence of personality traits (Sih et al., 2004) in these three beetles; however, this question will need to be more thoroughly investigated based on a larger number of individuals. From a conservation perspective, animal personality has important implications. Dispersal is often phenotype-biassed, with bolder and more exploratory individuals showing a higher dispersal tendency (Cote and Clobert, 2007; Cote et al., 2010). Such data could help explain colonisation dynamics (Cote et al., 2010) and the degree of success of species re-introduction (de Azevedo and Young, 2021).

4.3. Implications for the conservation of grassland beetle populations

This study highlights the limitations of using passive tags to track the movements of small terrestrial beetles. The fact that detection requires the antenna to pass over

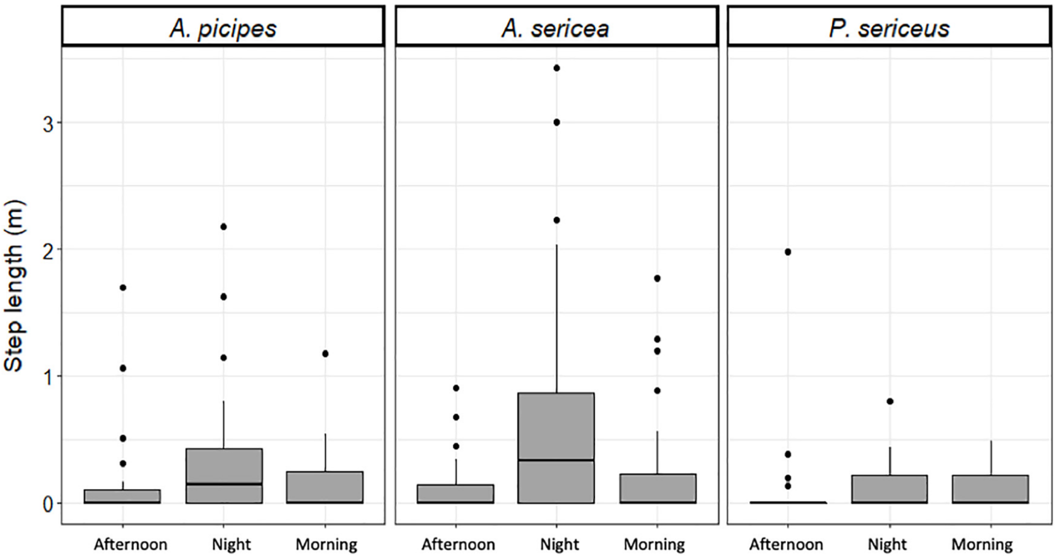


FIGURE 5
Step length per time of day for the three study species in the field.

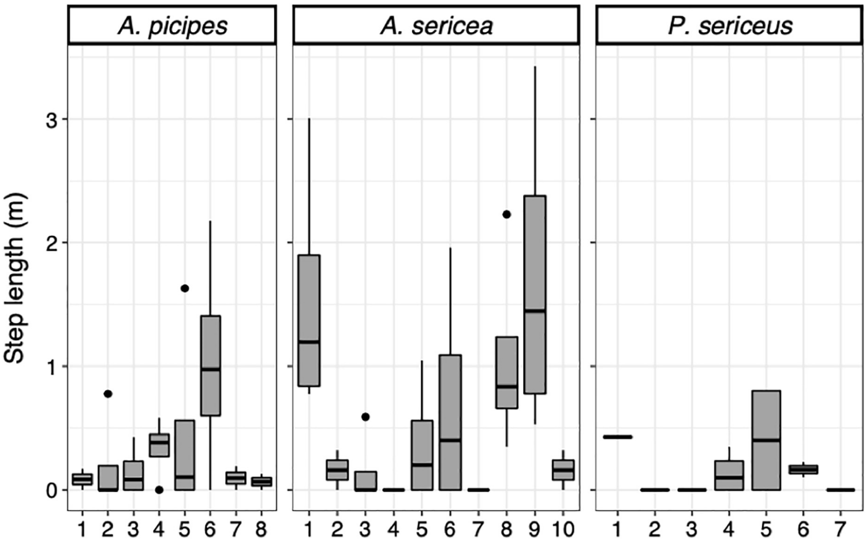


FIGURE 6
Step length in metre during night time per individual and species.

the tag makes it impossible to track individuals that cover several metres per day over several weeks, and thus to accurately identify their dispersal capabilities and home range. However, the data collected here provide valuable information on the biology of the three species, such as their nocturnal activity.

We were also able to determine their daily movements, which might have implications for their conservation and restoration in the study grassland. This dry grassland has been degraded by years of cultivation requiring the removal of the original stone cover (Buisson and Dutoit, 2006). Stones are a structuring factor for beetle communities (Blight et al., 2011), probably creating better microclimatic conditions (havens of moisture and low temperatures in summer, Lamb and Chapman, 1943; Nobel et al., 1992). These daily movement data shed light on the dynamics of natural colonisation of degraded areas from the original habitat. *Asida sericea* and *P. sericeus*, the two most abundant species in the original ecosystem, remain present in the formerly cultivated areas but in significantly lower abundance (Fadda et al., 2007; Blight et al., 2011). Although both ground-dwelling species move relatively short distances in the course of a day, they do move, confirming the hypothesis put forward by Fadda et al. (2008) of a possible natural colonisation of degraded areas from the nearby original habitat. Indeed, in the study area, degraded grasslands are contiguous with preserved grassland and there are no physical barriers such as concrete roads. The lower population densities found in formerly cultivated areas are therefore more likely due to less suitable habitats rather than to limited movement.

Knowing the rate of daily movement is also relevant for the implementation of effective restoration actions. One such proposed action is the restoration of stone cover, key for beetle communities. Even small stone patches dispersed randomly appear to be an effective way to increase beetle richness and abundance on formerly cultivated fields, as suggested by Blight et al. (2011) "several stone patches dispersed over a wide area maximises edge effects between the ecosystem to be restored and the restored habitat patches." However, the spatial distribution of the patches that will ensure the most efficient management depends on the beetles' movement capability. Our RFID monitoring suggests that this distance should not exceed 6 m, as 58% of individuals were found within 6 m of the release point 1 week later.

5. Conclusion

While we confirm the limitations of using RFID technology to track small ground-dwelling beetles over a long period in a dry Mediterranean grassland, the data collected provide interesting data on their biology, that may help in the development of restoration actions. Further work should address the challenge of tracking individuals' movements between restored habitat patches.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

OB, BG, and CA conceived the ideas. OB, BG, LM, and CA designed the experiment and collected the data. LM and CA analysed the data. OB and CA wrote the first draft. All authors contributed critically to the drafts.

Funding

This study was supported by the European Research Council, project ERC STG SCALED no. 949812.

Acknowledgments

We thank Nicolas Bachelier, Paul Guerra, Marine Rousseau, Hélène De Meringo, Victorine Demiralp, and Karolina Argote for their help in the field. We also thank Axel Wolf and the Eco-Musée de Crau for allowing us access to the Nature Reserve of Peau de Meau.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1040931/full#supplementary-material

References

Allen, A. M., and Singh, N. J. (2016). Linking movement ecology with wildlife management and conservation. *Front. Ecol. Evol.* 3:155. doi: 10.3389/FEVO.2015.00155

Batsleer, F., Bonte, D., Dekeukeleire, D., Goossens, S., Poelmans, W., Van der Cruyssen, E., et al. (2020). The neglected impact of tracking devices on terrestrial arthropods. *Methods Ecol. Evol.* 11, 350–361. doi: 10.1111/2041-210X.13356

Bérces, S., and Růžičková, J. (2019). Hungarian natural history museum. *Budapest Acta Zool. Acad. Sci. Hungaricae* 65, 335–348.

Blight, O., Fadda, S., Orgeas, J., Ponel, P., Buisson, E., and Dutoit, T. (2011). Using stone cover patches and grazing exclusion to restore ground-active beetle communities in a degraded pseudo-steppe. *J. Insect Conserv.* 15, 561–572. doi: 10.1007/s10841-010-9358-3

Boiteau, G., and Colpitts, B. (2001). Electronic tags for the tracking of insects in flight: effect of weight on flight performance of adult Colorado potato beetles. *Entomol. Exp. Appl.* 100, 187–193. doi: 10.1046/j.1570-7458.2001.00863.x

Boiteau, G., Vincent, C., Meloche, F., and Leskey, T. C. (2010). Harmonic radar: Assessing the impact of tag weight on walking activity of Colorado potato beetle, plum curculio, and western corn rootworm. *J. Econ. Entomol.* 103, 63–69. doi: 10.1603/EC09113

Buisson, E., Archibald, S., Fidelis, A., and Suding, K. N. (2022). Ancient grasslands guide ambitious goals in grassland restoration. *Science* 377, 594–598. doi: 10.1126/science.abo4605

Buisson, E., and Dutoit, T. (2004). Colonisation by native species of abandoned farmland adjacent to a remnant patch of Mediterranean steppe. *Plant Ecol.* 174, 371–384.

Buisson, E., and Dutoit, T. (2006). Creation of the Natural Reserve of la Crau: Implications for the creation and management of protected areas. *J. Environ. Manag.* 80, 318–326. doi: 10.1016/j.jenvman.2005.09.013

Cote, J., and Clobert, J. (2007). Social information and emigration: lessons from immigrants. *Ecol. Lett.* 10, 411–417.

Cote, J., Fogarty, S., Weinersmith, K., Brodin, T., and Sih, A. (2010). Personality traits and dispersal tendency in the invasive mosquitofish (*Gambusia affinis*). *Proc. Biol. Sci.* 277, 1571–1579. doi: 10.1098/rspb.2009.2128

de Azevedo, C. S., and Young, R. J. (2021). Animal personality and conservation: basics for inspiring new research. *Animals* 11:1019. doi: 10.3390/ani11041019

Devaux, J. P., Archiloque, A., BorelL, L., Bourrelly, M., and Louis-Palluel. (1983). Notice de la carte phyto-écologique de la Crau (Bouches du Rhône). *Biol. Écologie Méditerranéenne* 10, 5–54.

Fadda, S., Orgeas, J., Ponel, P., Buisson, E., and Dutoit, T. (2008). Conservation of grassland patches failed to enhance colonization of ground-active beetles on formerly cultivated plots. *Environ. Conserv.* 35, 109–116.

Fadda, S., Orgeas, J., Ponel, P., Buisson, E., Torre, F., and Dutoit, T. (2007). Past cultivation is a factor driving organization of dry grassland ground-active beetle communities. *Environ. Conserv.* 34, 132–139.

Gamer, M., Lemon, J., Fellows, I., and Singh, P. (2019). *irr: Various Coefficients of Interrater Reliability and Agreement. R package version 0.84.1*.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J. F., Aupinel, P., et al. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science* 336, 348–350.

Hernández, M. I. M. (2002). The night and day of dung beetles (Coleoptera, Scarabaeidae) in the Serra do Japi, Brazil: elytra colour related to daily activity. *Rev. Bras. Entomol.* 46, 597–600.

Hilderbrand, R. H., Watts, A. C., Randle, A. M., Hilderbrand, R. H., Watts, A. C., and Randle, A. M. (2005). The myths of restoration ecology. *Ecol. Soc.* 10:19.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6, 65–70.

Holyoak, M., Casagrandi, R., Nathan, R., Revilla, E., and Spiegel, O. (2008). Trends and missing parts in the study of movement ecology. *PNAS* 105, 19060–19065. doi: 10.1073/pnas.0800483105

Janowski-Bell, M. E., and Horner, N. V. (1999). Movement of the male brown tarantula, *Aphonopelma hentzi* (Araneae, Theraphosidae), using radio telemetry. *J. Arachnol.* 27, 503–512.

Jeannel, R. (1942). *Faune de France Coléoptères Carabiques. Fédération française des sociétés de sciences naturelles*, Paris: Lechevalier et fils, 573–1173.

Jepsen, N., Schreck, C., Clements, S., and Thorstad, E. B. (2005). "A brief discussion on the 2% tag/bodymass rule of thumb. Aquatic telemetry: advances and applications," in *Proceedings of the Fifth Conference on Fish Telemetry held in Europe*, (Ustica).

Kaláb, O., Musiolek, D., Rusnok, P., Hurtik, P., Tomis, M., and Koèárek, P. (2021). Estimating the effect of tracking tag weight on insect movement using video analysis: a case study with a flightless orthopteran. *PLoS One* 16:e0255117. doi: 10.1371/journal.pone.0255117

Kassambara, A. (2022). *Rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.1*.

Katzner, T. E., and Arlettaz, R. (2020). Evaluating contributions of recent tracking-based animal movement ecology to conservation management. *Front. Ecol. Evol.* 7:519. doi: 10.3389/FEVO.2019.00519/BIBTEX

Kenward, R. (2001). *A Manual for Wildlife Radio Tagging*. Cambridge, MA: Academic Press.

Kissling, D. W., Pattemore, D. E., and Hagen, M. (2014). Challenges and prospects in the telemetry of insects. *Biol. Rev.* 89, 511–530. doi: 10.1111/brv.12065

Koo, T. K., and Li, M. Y. (2016). Cracking the code: providing insight into the fundamentals of research and evidence-based practice a guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J. Chiopr Med.* 15, 155–163.

Lamb, J., and Chapman, J. E. (1943). Effect of surface stones on erosion, evaporation, soil temperature, and soil moisture1. *Agron. J.* 35, 567–578.

Le Gouar, P. J., Dubois, G. F., Vignon, V., Brustel, H., and Vernon, P. (2015). Demographic parameters of sexes in an elusive insect: implications for monitoring methods. *Popul. Ecol.* 57, 227–236.

Mallard, F., Le Bourlot, V., and Tully, T. (2013). An automated image analysis system to measure and count organisms in laboratory microcosms. *PloS One* 8:e64387. doi: 10.1371/journal.pone.0064387

Massol, F., Gravel, D., Mouquet, N., Cadotte, M. W., Fukami, T., and Leibold, M. A. (2011). Linking community and ecosystem dynamics through spatial ecology. *Ecol. Lett.* 14, 313–323.

Milanesio, D., Saccani, M., Maggiora, R., Laurino, D., and Porporato, M. (2016). Design of an harmonic radar for the tracking of the Asian yellow-legged hornet. *Ecol. Evol.* 6, 2170–2178. doi: 10.1002/ece3.2011

Moreau, M., Arrufat, P., Latil, G., and Jeanson, R. (2011). Use of radio-tagging to map spatial organization and social interactions in insects. *J. Exp. Biol.* 214, 17–21. doi: 10.1242/jeb.050526

Moskowitz, D., and May, M. (2017). Adult tiger spiketail (*Cordulegaster erronea* Hagen) habitat use and home range observed via radio-telemetry with conservation recommendations. *J. Insect Conserv.* 21, 885–895.

Negro, M. A., Casale, A. C., Migliore, L. U. C. A., Palestrini, C. L., and Rolando, A. N. (2008). Habitat use and movement patterns *Carabus olympiae*. *Eur. J. Entomol.* 5759, 105–112.

Nobel, P. S., Miller, P. M., and Graham, E. A. (1992). Influence of rocks on soil temperature, soil water potential, and rooting patterns for desert succulents. *Oecologia* 92, 90–96. doi: 10.1007/BF00317267

O'Neal, M. E., Landis, D. A., Rothwell, E., Kempel, L., and Reinhard, D. (2004). Tracking insects with harmonic radar: A case study. *Am. Entomol.* 50, 212–218. doi: 10.1093/ae/50.4.212

Peterson, R. A. (2021). Finding optimal normalizing transformations via bestnormalize. *R J.* 13, 310–329. doi: 10.32614/RJ-2021-041

Poli, C., Hightower, J., and Fletcher, R. J. (2020). Validating network connectivity with observed movement in experimental landscapes undergoing habitat destruction. *J. Appl. Ecol.* 57, 1426–1437.

R Core Team (2022). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Ramos-Fernández, G., Mateos, J. L., Miramontes, O., Cocho, G., Larralde, H., and Ayala-Orozco, B. (2004). Lévy walk patterns in the foraging movements of spider monkeys (*Ateles geoffroyi*). *Behav. Ecol. Sociobiol.* 55, 223–230.

Reynolds, D. R., and Riley, J. R. (2002). Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Comput. Electron. Agric.* 35, 271–307.

Riecken, U., and Raths, U. (1996). Use of radio telemetry for studying dispersal and habitat use of *Carabus coriaceus* L. *Ann. Zool. Fennici* 33, 109–116.

Rink, M., and Sinsch, U. (2007). Radio-telemetric monitoring of dispersing stag beetles: implications for conservation. *J. Zool.* 272, 235–243.

Römermann, C., Dutoit, T., Poschlod, P., and Buisson, E. (2005). Influence of former cultivation on the unique Mediterranean steppe of France and consequences for conservation management. *Biol. Conserv.* 121, 21–33.

Rùžièková, J., and Elek, Z. (2021). Recording fine-scale movement of ground beetles by two methods: potentials and methodological pitfalls. *Ecol. Evol.* 11, 8562–8572. doi: 10.1002/ece3.7670

Růžičková, J., and Veselý, M. (2016). Using radio telemetry to track ground beetles: movement of *Carabus ullrichii*. *Biologia* 71, 924–930.

Shrout, P. E., and Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychol. Bull.* 86, 420–428.

Sih, A., Bell, A., and Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends Ecol. Evol.* 19, 372–378. doi: 10.1016/j.tree.2004.04.009

Silcox, D. E., Doskocil, J. P., Sorenson, C. E., and Brandenburg, R. L. (2011). Radio frequency identification tagging: a novel approach to monitoring surface and subterranean insects. *Am. Entomol.* 57, 86–93.

Soldati, F. (2006). *Les asida latreille 1802 de la faune de France (col. tenebrionidae) rutilans*, ed. Les Presses Littéraires. Saint-Estéve: Les Presses Littéraires.

Talarico, F., Giglio, A., Pizzolotto, R., and Brandmayr, P. (2016). A synthesis of feeding habits and reproduction rhythm in Italian seed-feeding ground beetles (Coleoptera: Carabidae). *Eur. J. Entomol.* 113, 325–336.

Testud, G., Vergnes, A., Cordier, P., Labarraque, D., and Miaud, C. (2019). Automatic detection of small PIT-tagged animals using wildlife crossings. *Anim. Biotelemetry* 7:21. doi: 10.1186/s40317-019-0183-5

Thirgood, S., Mosser, A., Tham, S., Hopcraft, G., Mwangomo, E., Mlengeya, T., et al. (2004). Can parks protect migratory ungulates? the case of the Serengeti wildebeest. *Anim. Conserv.* 7, 113–120.

Vinatier, F., Chailleux, A., Duyck, P.-F., Salmon, F., Lescourret, F., and Tixier, P. (2010). Radiotelemetry unravels movements of a walking insect species in heterogeneous environments. *Anim. Behav*. 80, 221–229. doi: 10.1016/j.anbehav.2010.04.022

Appendix 1

Trajectories in the field for individuals that moved at least 1 m from their release point. Release points have been overlapped. Each individual is represented by a given colour. The white square shows their release point. Dots represent the position of the individuals during the 48 h and open circles the position after 1 week (when known). Dotted circles represent distances of 1 m and 6 m from the release point.

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 03 May 2023
DOI 10.3389/fevo.2023.1155885

OPEN ACCESS

EDITED BY
Thierry Dutoit,
UMR7263 Institut méditerranéen de biodiversité et d'écologie marine et continentale (IMBE), France

REVIEWED BY
Ádám Lovas-Kiss,
Hungarian Academy of Sciences, Hungary
Stephen Bonser,
University of New South Wales, Australia

*CORRESPONDENCE
Katalin Szitár
✉ szitar.katalin@ecolres.hu

RECEIVED 31 January 2023
ACCEPTED 14 April 2023
PUBLISHED 03 May 2023

CITATION
Szitár K, Tölgyesi C, Deák B, Gallé R, Korányi D and Batáry P (2023) Connectivity and fragment size drive plant dispersal and persistence traits in forest steppe fragments. *Front. Ecol. Evol.* 11:1155885.
doi: 10.3389/fevo.2023.1155885

Connectivity and fragment size drive plant dispersal and persistence traits in forest steppe fragments

Katalin Szitár[1]*, Csaba Tölgyesi[2], Balázs Deák[1,3], Róbert Gallé[1], Dávid Korányi[1] and Péter Batáry[1]

[1]'Lendület' Landscape and Conservation Ecology, Institute of Ecology and Botany, Centre for Ecological Research, Vácrátót, Hungary, [2]MTA-SZTE 'Momentum' Applied Ecology Research Group, University of Szeged, Szeged, Hungary, [3]'Lendület' Seed Ecology Research Group, Institute of Ecology and Botany, Centre for Ecological Research, Vácrátót, Hungary

Introduction: Land use changes have seriously fragmented grasslands leading to extensive biodiversity loss worldwide. Habitat fragmentation affects grasslands at both local and landscape scales, adversely affecting the probability of species colonisation and extinction. In our study, we addressed the effects of fragment size and landscape-scale habitat connectivity on the vegetation composition in two grassland types, i.e., loess steppe fragments (situated on kurgans) and sand steppe fragments of the Pannonian forest steppe region.

Methods: We collected frequency data on vascular plant species in 12 1 m×1m quadrats in altogether 60 fragments along a connectivity gradient in sand steppes and loess steppe fragments. We analysed whether habitat specialists, generalists and exotic species were affected by the level of fragmentation based on species richness and traits related to local persistence (life span, clonality and soil seed bank type) and dispersal (dispersal strategy and seed mass) in the two grassland types using linear mixed-effects models.

Results: Based on our results, both fragment size and landscape-scale connectivity were important in shaping the trait composition of the vegetation. We observed more fragmentation effects in generalist than in specialist species. We found that isolation resulted in fewer specialist species with autochorous dispersal strategy in loess steppe fragments, but, at the same time, also fewer exotics. Isolated loess steppe fragments harboured fewer generalist species with persistent seed bank. Large loess steppe fragments supported more wind-dispersed species than smaller ones. In isolated sand steppe fragments, generalists were more frequent with endozoochorous dispersal strategy and without clonal propagation. Life span, clonal propagation and seed mass did not depend on the level of fragmentation in the two grassland types.

Discussion: Our results imply that both sand and loess steppe fragments can rely to some extent on the persistence of clonal perennial specialist and generalist species in small and isolated patches to mitigate fragmentation effects. In conclusion, these processes should be supported by the preservation of large fragments, increase of habitat connectivity combined with targeted management of exotic species.

KEYWORDS

adventive species, functional trait, habitat specialist, habitat isolation, terrestrial habitat island, kurgan, Pannonic loess steppic grassland, Pannonic sand grassland

1. Introduction

Land use change is one of the primary drivers of biodiversity loss worldwide, causing degradation and reduced habitat availability (Isbell et al., 2022). Temperate grasslands represent exceptionally high conservation values as they are among the most species-rich communities when considering small ($<50\,m^2$) spatial scales (Wilson et al., 2012). However, they have been increasingly fragmented due to ploughing and afforestation (Habel et al., 2013). Despite the drastic level of habitat loss, grasslands still contribute significantly to global and local biodiversity by harbouring many endangered animal and plant species confined to these habitats (Squires and Feng, 2018). Species preservation is essential to provide an insurance biodiversity capacity to reduce the risk caused by the temporal variability of ecosystem processes under current global changes (Loreau et al., 2001).

Habitat fragmentation may affect species at both local and larger spatial scales (Auffret et al., 2018). At the landscape scale, the compositional and configurational heterogeneity of the surrounding habitats shape the species pool by affecting diaspore flow (Gaujour et al., 2012). At the local scale, species pool is filtered primarily by altered abiotic conditions and species interactions (Maurer et al., 2003). Increasing fragmentation leads to an increased distance among habitat patches as it can considerably limit dispersal processes (Taylor et al., 1993) and reduce gene flow (Heinken and Weber, 2013). At the local scale, fragmentation results in reduced habitat patch sizes (Uroy et al., 2019) increasing the extinction risk of species populations due to reduced habitat quality and stronger edge effects (Soons et al., 2005). Unpredictable catastrophic events and demographic stochasticity can also lead to population extinctions more frequently in small patches (Turner, 1996). As isolation and fragment size reduction can amplify each other's effect (Didham et al., 2012), we can gain deeper insights with their joint assessment.

Functional plant traits are effective tools for investigating fragmentation effects offering more general and mechanistic insights into ecosystem processes and higher predictability than taxonomic approaches (McGill et al., 2006). Colonisation and extinction processes cause direct changes in community composition in the course of fragmentation (Jackson and Sax, 2010). These processes are driven by species-specific functional traits linked to population biological processes (Janečková et al., 2017). We might expect that species with efficient propagule dispersal or local persistence traits are more successful and well-preserved in habitat fragments than species without these traits (Marini et al., 2012).

Dispersal potential can be best captured by assessing the main dispersal modes and propagule characteristics of the species, such as seed mass, shape and size (Ottaviani et al., 2020). The long-distance dispersal ability is critically important for species survival in fragmented landscapes (Bacles et al., 2006). Species dispersing by wind or animals efficiently are assumed to be able to colonise more isolated fragments (Vittoz and Engler, 2007). Species with small seed mass have higher dispersal potential by wind because light seeds have lower terminal velocity (Lindborg et al., 2012), whereas heavy seeds with appendices may disperse by animals more effectively (Amartuvshin et al., 2019). Plant species with high dispersal potential are expected to be less vulnerable to fragmentation (Eriksson, 1996), although some studies detected similar (Hemrová and Münzbergová, 2015) or even higher sensitivity to fragmentation of long-distance than short-distance dispersers (Deák et al., 2018).

Long life span, clonal reproduction and persistent soil seed bank are the main traits that are likely to contribute to local persistence (Maurer et al., 2003). Long-lived species are assumed to be less susceptible to fragmentation because of their smaller fluctuations in abundance (Heinken and Weber, 2013). Species capable of clonal propagation with a potentially unlimited life span are not dependent on sexual reproduction for population survival; therefore, they are expected to be less impacted by isolation (Heinken and Weber, 2013). However, environmental suppression of sexual propagation can also lead to monoclonal populations lowering long-term population viability (Honnay and Bossuyt, 2005). The persistent seed bank in the soil also enables plant populations to survive disturbances and tolerate environmental fluctuations, thus prolonging species persistence in the community (Eriksson, 1996). Based on the contradictory results of the different case studies, fragmentation effects on plant traits seem to be context and habitat specific, highlighting the need to assess them in various ecosystems (Heinken and Weber, 2013).

Inspecting only total species richness patterns can hide important aspects of underlying mechanisms of responses to fragmentation (Lindborg et al., 2012). Depending on habitat specialisation, fragmentation effects vary on plant species (Yan et al., 2022). Specialist species have narrow environmental tolerances and thus are limited to specific habitat types (Pandit et al., 2009), and are more susceptible to disturbances than generalist species (Vázquez and Simberloff, 2002). Habitat generalists are important grassland constituents contributing to the total richness and abundance of the vegetation but do not indicate directly good or bad ecological status (Deák et al., 2020). Generalists can persist under a wide range of abiotic conditions; therefore, they are assumed to be less affected by fragmentation (Yan et al., 2022). Non-indigenous species may behave similarly to generalists but have exceptionally high practical importance as drivers of unfavourable ecosystem changes and degradation indicators (Didham et al., 2005). In this paper, we used species groups based on habitat specialisation and origin to understand how the functional composition of the vegetation changes due to fragmentation.

The forest-steppe fragments of the Pannonic biogeographical region provide an ideal setting to assess the effects of fragmentation. The region is the westernmost part of the Eurasian forest-steppe biome, a distinct vegetation zone between closed forests and steppes in subhumid-semi arid climate zones (Erdős et al., 2018a,b). The vegetation is of a mosaic nature where both the forest and steppe are stable vegetation components (Fekete et al., 2002). Two substrate types, sand and loess, are widely distributed in the region, and both harbour valuable habitats listed as Natura 2000 priority habitat types (Pannonic loess steppic grasslands and Pannonic sand steppes) in Annex I of EU's Habitat Directive (European Commission, 2013). Loess and sand steppes represent two extremes in terms of abiotic conditions and productivity (Molnár et al., 2012). Sandy substrates create highly stressed abiotic conditions, whereas water and nutrient cycling is more favourable for plant life on loess (Fekete et al., 2002). Loess and sand steppes have been exposed to extensive anthropogenic habitat loss and fragmentation (Gallé et al., 2022). Because of their clearly defined borders and unsuitable matrix, they can be regarded as terrestrial habitat islands (Deák et al., 2016), serving as excellent model systems to study the effects of fragmentation (Ottaviani et al., 2020).

In our study, we tested if species groups with different origin and habitat specialisation (i.e., specialists, generalists and exotics) show general response patterns against fragmentation in two contrasting steppe types of the Pannonian forest steppe region. We focused on the vegetation of open sand steppe and loess steppe fragments on forest steppes and kurgans (ancient burial mounds) in the Great Hungarian Plain. We assessed the effects of fragment size (large vs. small fragments) and landscape-scale connectivity (Hanski's connectivity index) on the richness and frequency of species with traits related to local persistence (life span, clonal propagation and soil seed bank type) and propagule dispersal (dispersal strategy and seed mass). We expected that (i) specialist plant species would experience more significant effects of connectivity and fragment size than generalists or exotics because they are more dependent on the fragmented steppe habitats; (ii) the importance of connectivity would be higher for dispersal-limited species and (iii) fragment size would affect local persistence trait distribution primarily.

2. Materials and methods

2.1. Study area

Our study region lies in the Southern part of the Great Hungarian Plain (Figure 1). This is a lowland region with a continental climate characterised by mean annual temperature of 11°C and mean annual precipitation of 550 mm. The semi-natural vegetation of the Pannonic forest-steppe zone can be characterised by a mosaic of dry and open oak (*Quercus* spp.), and poplar (*Populus* spp.) forests and *Festuca* and *Stipa* species dominated steppes (Molnár et al., 2012). Here we focused on two major grassland components of the vegetation, i.e., open sand steppe and loess steppe.

Open sand steppes are endemic communities (*Festucetum vaginatae*) to the Pannonic biogeographical region. They are semi-desert-like perennial grasslands occurring in inland sand dune ridges. The average cover of vascular plants is relatively sparse, ranging between 40 and 70% (Erdős et al., 2018a,b). The rest of the surface is usually covered by cryptogams (lichens and mosses) and bare ground. Tussock-forming *Festuca vaginata* and *Stipa borysthenica* dominate the vegetation, with many rare and threatened forbs confined to this vegetation type (Csecserits et al., 2011). The soil is wind-blown arenosol with extremely low (below 3%) humus content and low water-retention capacity (Csecserits et al., 2011). Because of the low productivity of the soil, open sand steppes were mostly grazed by sheep or remained unmanaged until the 19th century (Molnár et al., 2012). In the 1920s, intensive lowland afforestation programmes started, and by the end of the 20th century, most of the open sand grasslands were converted to non-native tree plantations in the region (Biró et al., 2008).

Loess steppes (*Salvio-Festucetum rupicolae*) are highly productive perennial vegetation types developed on chernozem soil (Fekete et al., 2002). The vegetation is diverse, dominated by several perennial grasses (e.g., *Festuca rupicola*, *Brachypodium pinnatum* and *Stipa* spp.) and are rich in threatened forbs (Deák et al., 2016). Because of their favourable edaphic conditions and flat topography, loess steppe transformation most probably started already in the Neolithic, and was accelerated from the 18th century due to agricultural expansion and intensification (Deák et al., 2021a,b). It resulted in the loss of 90% of their original area by the end of the 20th century (Deák et al., 2016). In this study, we focused on kurgans, i.e., ancient burial mounds created in the IV-I millennia BC (Deák et al., 2016). Kurgans usually take on a hemispherical shape with a height ranging between 0.5 and 15 m, and a diameter between 10 and 110 m (Dembicz et al., 2021). As most kurgans are small and isolated in the region, they are usually left unmanaged resulting in the survival of semi-natural vegetation but also unfavourable spontaneous shrub encroachment and litter accumulation (Deák et al., 2016).

The sand and loess steppe fragments of our study could be characterised by contrasting surrounding landscape (Figure 1). The sand fragments were embedded into the matrix of small-scale plantation forests planted with non-native *Pinus sylvestris, P. nigra* and *Robinia pseudoacacia* trees, and some native *Populus canescens* (Biró et al., 2008). These were even-aged plantation forests created after ploughing and harvested with clear-cutting (Csecserits et al., 2016). The loess fragments were surrounded by intensively used large-scale agricultural fields, with some farmhouses and pastures. The region's main crops are winter cereals, maize, sunflower and alfalfa (Gallé et al., 2022).

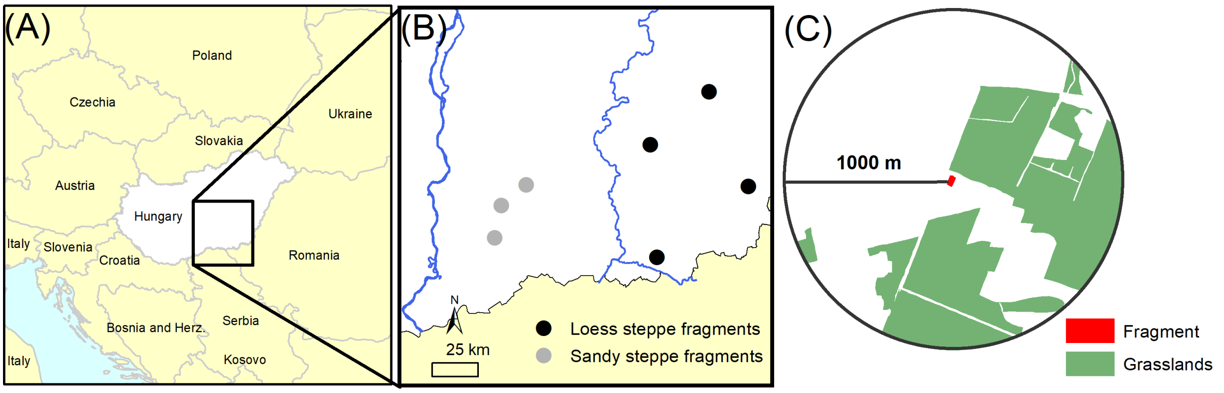


FIGURE 1
(A) Map of Central Europe showing Hungary, **(B)** map of the Great Hungarian Plain in Southeast Hungary with the study sites and **(C)** a loess fragment study site with the 1,000m-buffer that was used to estimate Hanski's connectivity. Note that for sand steppe fragments a 500m-buffer was used.

2.2. Study sites

We selected 15 small and 15 large fragments for each steppe type along a connectivity gradient (Figure 1). We considered fragment size as a patch-scale characteristic and connectivity as a landscape-scale predictor. We visited 160 fragments around three (Fischerbócsa, Kéleshalom and Pirtó) and four villages (Dévaványa, Kunágota, Makó and Szentes) for sand and loess steppes, respectively. The available fragment sizes differed in the two types; therefore, we applied differing size ranges for the selection. For sand steppes, the size of the small and large fragments ranged between 0.16 and 0.48 ha, and 0.93–6.88 ha, respectively. In the case of loess steppes, the size of the small and large fragments varied between 0.01–0.10 ha, and 0.20–0.44 ha, respectively.

For measuring connectivity, we calculated Hanski's connectivity index (Hanski and Ovaskainen, 2000) for all potential fragments:

$$CIi = \sum_{j \neq i} \exp\left(-\alpha d_{ij}\right) A_j^{\beta}$$

where d_{ij} stands for the shortest edge-to-edge distance (in km) between fragment *i* and *j* within the search radius, A_j is the size of habitat patch *j* (in m^2), α is a constant scaling the species-specific dispersal ability over distance d_{ij} and β is an emigration parameter scaled over the size of patch *j* as a source of migration (Moilanen and Hanski, 2001). Following Rösch et al. (2013), both parameters were set to 0.5 as we applied the index to entire communities. The grain size of the landscape matrix differed in the two steppe types, with a small-scale forest plantation matrix in the first, and a large-scale agricultural matrix in the latter case (Kuli-Révész et al., 2021). Therefore, we applied different buffer radii for the connectivity index calculations (500 for sand and 1,000 m for loess steppes) based on the ecosystem base map of Hungary (Tanács et al., 2021) and Google aerial photographs by using Quantum GIS 3.6.1 (QGIS Development Team, 2019). We considered all semi-natural vegetation patches (open sand grasslands, loess steppe, other forest-steppe fragments and alkali grasslands) as sources of immigration. The connectivity values ranged between 24 and 811 for sand steppe (mean = 394, SD = 206), and from 0 to 2,637 for loess steppe fragments (mean = 689, SD = 748).

2.3. Vegetation sampling

We sampled the vegetation in 12 1 × 1 m quadrats in each fragment. Six quadrats were placed 1–2 m from fragment edges, and six were positioned in the centre to cover habitat heterogeneity. They were located at least 5 m from each other to avoid spatial autocorrelation. We recorded the presence of each vascular plant species in the quadrats in May–June 2019 and 2020. We summed up the occurrences of each plant species for all quadrats in the two sampling periods for each fragment. Thus, the frequency of a species (used as a proxy for abundance) could range between zero and 24 in a fragment.

We divided the plant species according to their habitat specialisation into the following categories: (i) steppe specialists with a regional preference for open sand or loess steppes; (ii) generalist species including all other native species not confined exclusively to sand or loess steppe habitats and (iii) exotic species. Species were assigned to groups based on field guides and expert knowledge (Király, 2009; Gallé et al., 2022). We assessed five traits related to local persistence and dispersal potential to reveal whether functional traits of the three species groups respond differently to local- and landscape-scale fragmentation effects in the two grassland types. We chose traits that are rather stable within species because we collected trait values from databases that were not observed in the field.

For local persistence, these were the following: life span, clonal propagation and soil seed bank longevity. Life span was categorised into two levels, short-lived species, including annuals and biennials, and long-lived species. The life span of some species could be both short and long. For these, we used the most common life span category according to Király (2009). Clonality was used as a categorical trait with two values (clonal and non-clonal). We differentiated between transient and persistent soil seed bank types, where transient stands for longevity shorter than 1 year and persistent longer than a year, including short- and long-term persistent seed banking. In cases where more than one value was available, we assigned the longer term value for the species. We used seed dispersal strategies as a functional trait related to diaspore dispersal potential, according to Sádlo et al. (2018). They described dispersal mode combinations with different relative importance corresponding to similar diaspore morphological features and ecological demands of the species. They characterised nine strategies based on dispersal mode combinations. In our fragments, four strategy types occurred [dispersal strategy names follow the nomenclature suggested by Sádlo et al. (2018)]: (i) *Allium* type was the most common strategy with mainly autochory and to a small degree epi-, endozoochory and anemochory; (ii) *Bidens* type is dominated by autochory and epizoochory, and to a lesser extent endozoochory; (iii) in *Cornus* type, species disperse predominantly with autochory and endozoochory and (iv) *Epilobium* type can be characterised by anemochory beside autochory, and rarely zoochory. Seed mass has an important role in both local persistence and dispersal potential, and we considered it in relation to both processes. Seed mass was measured as thousand seed weight. Trait values were collected from various databases such as PADAPT (Sonkoly et al., 2022), Pladias (Wild et al., 2019), LEDA (Kleyer et al., 2008), CLO-PLA (Klimešová and de Bello, 2009), TRY (Kattge et al., 2020) and regional seed mass records (Csontos et al., 2003, 2007; Török et al., 2013, 2016), and the field guide of the Hungarian flora (Király, 2009). For details of species list and trait values see Supplementary Table 1.

2.4. Statistical analyses

We analysed the effects of fragment size and connectivity and their interaction on the richness of the three species groups (i.e., specialists, generalists and exotics) and the richness and frequency of plant species with different trait categories. Dispersal strategy categories with less than three species were not tested statistically (i.e., *Bidens* type and *Cornus* type specialists and exotics except for *Cornus* type exotics in loess steppe fragments). For each fragment, we calculated the number and frequency of species assigned to each species group and trait category. We also quantified the fragment-level community-weighted mean for the seed mass weighted by species frequency. Statistical analyses were performed using R version 4.1.1 (R Core Team, 2022). We implemented the analyses for the two grassland types separately. We modelled the relationships with generalised linear mixed-effects models (GLMMs) using Poisson error terms and log link function for the categorical traits (Bates et al., 2021) and linear mixed-effects models for the continuous variable, community-weighted mean of seed mass after log transformation (Pinheiro et al., 2017). Hanski's connectivity index values were log-transformed and ranged between 0 and 1. We treated fragment size as a categorical fixed variable (small vs. large), the ranged connectivity index as a continuous explanatory variable and location (village) served as a random factor in the models. The models were visually inspected for violations of homoscedasticity by checking residuals against fitted values. We tested the models for overdispersion and zero inflation using the DHARMa package (Hartig, 2022). When we detected overdispersion, we recalculated the models with negative binomial error term. In the case of significant zero-inflation, we refitted the model as a zero-inflated Poisson or negative binomial model using fragment size as the explanatory variable in the zero part of the model, while fragment size and connectivity and their interaction were used in the non-zero part of the models using the glmmTMB package (Brooks et al., 2017). We calculated the conditional coefficient of determination (i.e., variance explained by the entire model including both the fixed and random effects) for the models via the delta method with the MuMIn package (Barton, 2022). Data were visualised using the ggplot2 package (Wickham, 2011).

3. Results

We recorded a total of 178 species in the sand steppe fragments and 270 species in the loess grassland fragments during the 2 years of sampling (Table 1). Sandy fragments harboured 67 specialists, 97 generalists and 14 exotic species, while loess grassland fragments were more diverse, with altogether 43 specialist, 193 generalist and 34 exotic species. The mean richness was 22.3 (SD = 3.9) for specialist, 32.7 (SD = 7.9) for generalist and 4.4 (SD = 1.7) for exotic species in the sand steppe fragments, and 4.4 (SD = 2.9), 54.3 (SD = 8.0) and 5.5 (SD = 2.7) in the loess steppe fragments, respectively. We found that the specialist species pool was dominated by perennial and clonal species as opposed to generalist species in both grassland types (Table 1). In both grassland types, species with a persistent seed bank and autochory-dominated *Allium*-type dispersal strategy dominated the vegetation in every species group. The average seed mass was the lowest in the case of specialists and the highest in exotics in both grassland types.

In the sand steppe fragments, we found no effect of either fragment size or connectivity on the richness of the three species groups (Supplementary Table 2). In contrast, the species richness of specialist and exotic species was higher with 69 and 31% in the most connected fragments compared to the most isolated ones in loess steppe fragments regardless of their size (Figures 2A,B).

We did not find any significant effect of connectivity and fragment size on the richness or the frequency of short-lived, long-lived or clonal species in any species groups of the two steppe types (Supplementary Tables 3, 4). In contrast, isolated sand steppe fragments had a higher richness of non-clonal generalists than connected patches (Figure 3A). In the case of loess steppe specialists, we found that non-clonal species were more abundant in smaller fragments than in the larger ones (Figure 3B). Exotic species did not show any significant pattern in clonality along connectivity gradient or fragment size in either of the grassland types (Supplementary Table 4). We did not observe any effects of connectivity or fragment size on the richness or frequency of specialists with different soil seed bank persistence in either fragment type (Supplementary Table 5). We found that both generalist and exotic species forming persistent seed bank were more frequent in well-connected loess fragments (Figures 3C,D). This difference was not observed in the case of sand steppe fragments. Species with transient seed bank did not show any trend along the connectivity gradient or fragment size in either species groups or grassland types (Supplementary Table 5).

Species with *Allium* and *Epilobium* dispersal strategies (i.e., autochory and anemochory) dominated both grasslands with altogether 71% and 17%, and 77% and 11% in sand and loess fragments, respectively. Autochorous *Allium*-type species did not respond to either connectivity or fragment size in our study, except for loess fragments, where increased connectivity resulted in a higher richness of *Allium*-type specialists (Supplementary Table 6; Figure 4A). Isolation resulted in a higher frequency of generalist species with autochory- and endozoochory-dominated *Cornus*-type dispersal in smaller compared to larger fragments in the sand steppes (Figure 4B). We observed more species and higher frequency of generalists *Epilobium* dispersal strategy characterised by anemochory and autochory in large than in small loess steppe fragments (Figures 4C,D). We showed that *Cornus*-type exotic species were more abundant in terms of both richness and frequency in small compared to large isolated fragments (Figures 4E,F). *Bidens* dispersal type with autochory and epizoochory dispersal modes was not different based on fragment size and connectivity in either species group and fragment type (Supplementary Table 6). Community-weighted means of seed mass did not have any trend along the connectivity gradient or with increasing fragment size for either species group in the two grassland types (Supplementary Table 7).

4. Discussion

Our results highlighted that both local and landscape-scale factors were important in shaping the vegetation composition of Pannonic steppe fragments. We observed more fragmentation effects in generalists than in specialist species. We found that isolation resulted in fewer specialist species with autochorous (*Allium* type) dispersal in loess steppe fragments. Isolated loess steppe fragments also harboured fewer generalist species with persistent seed bank but, at the same time, also fewer exotics. Large loess steppe fragments supported more species with *Epilobium*-type dispersal strategy than smaller ones. We did not find any systematic change along the two fragmentation factors in the sand fragments, except for the high frequency of generalists without clonal propagation and *Cornus*-type (endozoochory- and autochory-dominated) dispersal strategy in small and isolated fragments.

4.1. Fragmentation effects on species groups

In sand steppes, we did not observe any fragmentation effects on the trait composition of the specialist flora. This was contrary

TABLE 1 Number of species occurred in each species groups, percentage of species assigned to each category of local persistence and dispersal potential traits, and average thousand seed weight of the species for each species groups found in sand and loess steppe fragments.

	Sand steppe fragments			Loess steppe fragments		
	Specialist	Generalist	Exotic	Specialist	Generalist	Exotic
Number of species	67	97	14	43	193	34
Life span						
Long-lived (%)	91.0	42.3	64.3	86.0	36.8	38.2
Short-lived (%)	9.0	57.7	35.7	14.0	63.2	61.8
Clonality						
Clonal (%)	61.2	36.1	50.0	51.2	31.1	29.4
Non-clonal (%)	38.8	63.9	50.0	48.8	68.9	70.6
Soil seed bank persistence						
Persistent (%)	50.7	62.9	50.0	55.8	71.5	70.6
Transient (%)	17.9	25.8	21.4	18.6	11.4	11.8
No data (%)	31.3	11.3	28.6	25.6	17.1	17.6
Dispersal strategy						
Allium type (%)	80.6	68.0	42.9	90.7	76.2	61.8
Bidens type (%)	0	7.2	7.1	0	8.3	5.9
Cornus type (%)	1.5	10.3	14.3	0	4.7	20.6
Epilobium type (%)	17.9	14.4	35.7	9.3	10.9	11.8
Mean seed mass (mg/1000 seeds + SD)	2.1 (3.9)	6.1 (22.8)	30.8 (58.5)	1.6 (1.7)	5.0 (16.2)	31.2(68.4)

to our expectations, as several studies showed that habitat specialists are sensitive to fragmentation (e.g., Auffret et al., 2018; Yan et al., 2022). The absence of an effect of sand steppe fragmentation on the distribution of specialists may refer to their better dispersal abilities (Hemrová and Münzbergová, 2015), long-term species persistence (Tilman et al., 1994) or their combination. This is in line with our results, as specialist species of the sand steppes have a high potential for local persistence because the majority of the species are long-lived clonal perennials and have lighter seeds helping dispersal among remnant patches (Bakker et al., 1996).

We observed more fragmentation effects in generalists and exotic species than in specialists. Rösch et al. (2013) found that community composition changes were primarily driven by the responses of generalist species during the fragmentation of dry calcareous grasslands as they compose the dominant part of the vegetation. Pandit et al. (2009) also suggested that generalists should respond strongly to patch configuration and connectivity gradients. In line with them, we observed most habitat fragmentation effects in the case of the generalist species group. We found that clonality and dispersal strategies of generalist species depended on fragment size, connectivity or both factors in the two grassland types, and soil seed bank persistence in loess fragments. Reduced patch size results in a high edge-core area ratio that can be exploited by generalists as opposed to specialists (Brückmann et al., 2010). In such case, habitat generalists offer insurance biodiversity, especially if the habitat is disturbed (Deák et al., 2020). However, the increasing dominance of generalist species following fragmentation relative to specialists can lead to biotic homogenisation and be considered an unfavourable degradation process (Öckinger et al., 2010).

We detected a two-fold frequency increase of exotic species in loess steppe fragments along the increasing connectivity gradient, but we found no such effect in sand fragments. This result suggests that exotic species in the sand steppes are mostly good dispersing invasives that are not hindered by fragmentation (Csecserits et al., 2016), while the exotics of loess remnants are dispersal limited to some extent. Moreover, forest plantations around sand fragments also facilitate the invasion of alien plants (Szitár et al., 2014). We found differential responses of the species groups, suggesting that focusing on only one group (e.g., specialists) may be insufficient to evaluate vegetation responses to habitat fragmentation.

4.2. Fragmentation effects on dispersal traits

Local species persistence and dispersal traits have been shown to influence plant species sensitivity to habitat fragmentation by several studies (e.g., Lindborg, 2007; Marini et al., 2012). Plant species with high dispersal potential are expected to be disproportionally overrepresented in isolated patches (Dupré and Ehrlén, 2002). We found that not only isolation but also fragment size affected dispersal strategies according to Sádlo et al. (2018). Endozoochorous dispersal (*Cornus* type) strategy was more frequent in small and isolated sites among sand generalists and exotics in loess fragments. Most of these species were shrubs and trees with efficient bird dispersal (e.g., *Celtis occidentalis*, *Elaeagnus angustifolia*, and *Morus alba*). Extensive shrub encroachment is considered unfavourable in this region as they take up the space from the herbaceous vegetation (Ónodi et al., 2021), and if they are

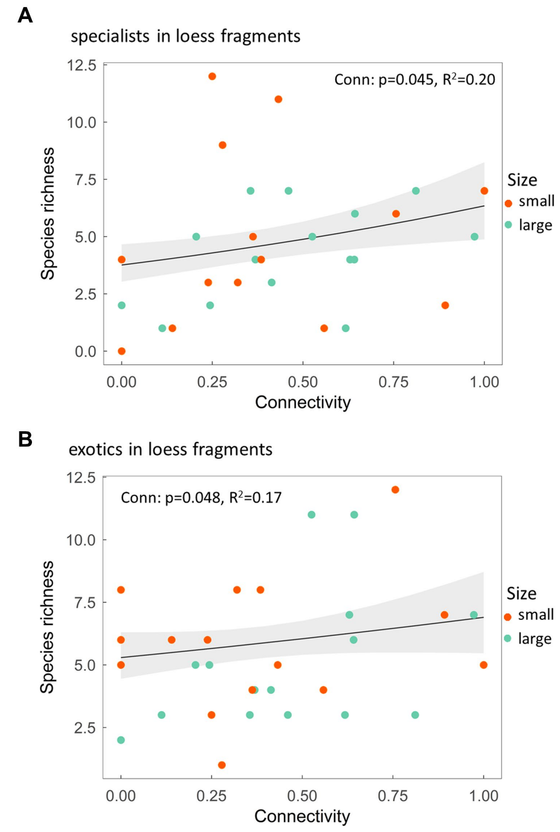


FIGURE 2
(A) Species richness of specialist and **(B)** exotic species along a connectivity gradient in loess steppe fragments. *p*-value shows the statistical significance of the explanatory variable, R^2 stands for the conditional coefficient of determination of the model, whereas the grey shaded area represent the standard error of the mean.

non-native, transformative vegetation changes and biotic homogenisation can occur (Didham et al., 2005). Generalists dispersing predominantly with anemochory and autochory (*Epilobium* dispersal strategy type) in loess fragments were more species rich and frequent in large fragments showing that successful arrival and establishment by wind is proportional to habitat area; therefore, this strategy is less efficient in small remnants. We did not find any fragmentation impacts for the epizoochory- and autochory-dominated *Bidens*-type dispersal strategy, maybe because of the general absence of grazing in both ecosystem types, similar to the results of Soons et al. (2005). In fragmented landscapes, many habitat remnants can remain unoccupied by bad disperser species (Lindborg et al., 2012). In line with this, we found that isolation also negatively affected loess specialist species with autochorous *Allium-type* dispersal. This has important nature conservation implications as these species have the least efficient autochorous dispersal (Vittoz and Engler, 2007). Therefore, they are particularly sensitive to fragmentation and may need active interventions to mitigate dispersal limitation or species reintroduction.

Propagule size is an important functional trait for both species persistence and dispersal, as small diaspores are expected to have higher colonisation potential (Marini et al., 2012), whereas longer longevity but also higher local colonisation rates are predicted for larger ones (Fröborg and Eriksson, 1997). However, similar to our results, seed mass was not always found to be correlated with remnant area or isolation (Dupré and Ehrlén, 2002; Lindborg, 2007).

4.3. Fragmentation effects on local persistence traits

Species longevity, the presence of persistent seed bank and clonal reproduction represent a potentially important form of temporal functional connectivity extending the life span of remnant populations and rescuing those that have gone locally extinct (Auffret et al., 2015). We did not find any fragmentation impact on the longevity of plant species in our study. This was opposing to our expectations, as several studies proved that perennial species are less sensitive to habitat loss because of their long generation time (e.g., Heinken and Weber, 2013), whereas short-lived species are more susceptible to fragmentation, especially fragment size, because of their larger natural fluctuations in abundance (Marini et al., 2012).

Fragmentation is expected to affect clonally reproducing species less than non-clonal species (Hemrová and Münzbergová, 2015). According to that, we did not observe any response of species with the ability of clonal propagation against fragmentation. However, we found that non-clonal species were more abundant in isolated and small patches in both sand and loess fragments. Likewise, Lindborg (2007) observed that long-lived species with clonal ability were unrelated to contemporary grassland connectivity and area whereas non-clonal species were more abundant in isolated fragments. However, they also found that small and isolated fragments in the 50 years' historical landscape had a high share of species with clonal propagation, inferring that an extinction debt existed in clonal plants and may exist in our case, too (Tilman et al., 1994).

The persistent seed bank can create a temporal rescue effect for communities (Honnay et al., 2008). We did not find any association of fragmentation with seed bank persistence in sand fragments. Lindborg (2007) suggested that in the long term, connectivity loss and area reduction would create a plant community dominated by long-lived and clonal plants and plants with a persistent seed bank. In the case of loess fragments, this potential seems to be depleted as we found a lower frequency of generalists with persistent seed bank in isolated sites.

4.4. Fragmentation effects on the two grassland types

Overall, the vegetation of loess steppe fragments seemed to be more affected by fragmentation than that of the sand steppe fragments, although we did not make a direct comparison of the two grassland types. In sand steppe fragments, the taxonomic richness of the species groups was similar in fragments with different sizes and levels of connectivity. In contrast, specialist and exotic species occurring in loess fragments were more species rich in connected fragments. This difference was most probably due to the smaller size of loess fragments and their functionally more hostile large-scale agricultural matrix compared to sand fragments (Gallé et al., 2022). Furthermore, we can consider sand fragments functionally less isolated because there are more road verges, forest fringes (Rédei et al., 2014), and thin grassland strips between forestry units that can serve

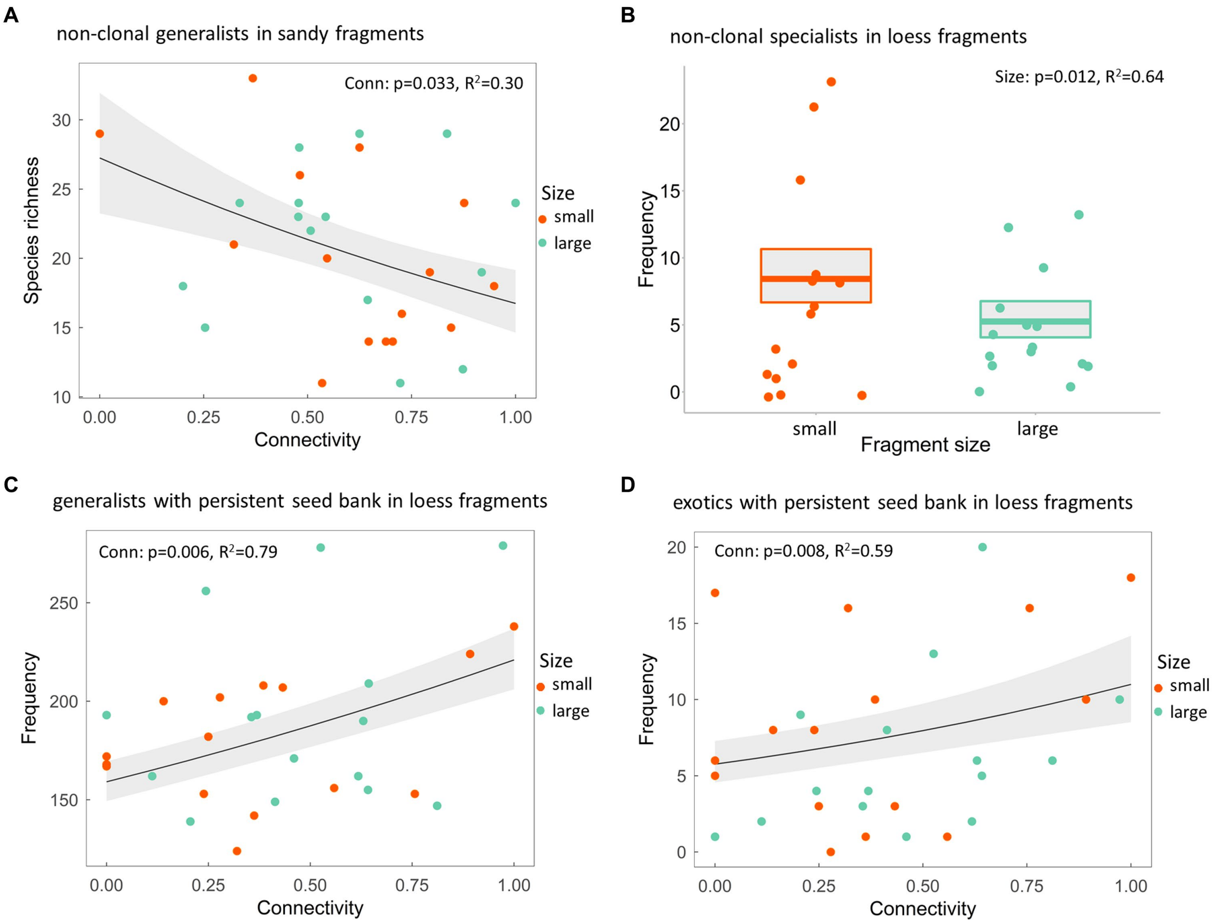


FIGURE 3
(A) Species richness of non-clonal generalists in sand grassland fragments, **(B)** frequency of non-clonal specialists, **(C)** generalists with persistent seed bank and **(D)** exotics with persistent soil seed bank in loess steppe fragments in small and large fragments along a connectivity gradient. *p*-value shows the statistical significance of the explanatory variable, R^2 stands for the conditional coefficient of determination of the model, whereas the grey shaded area represent the standard error of the mean.

plant population persistence by supporting meta-population dynamics and thus immigration processes (Szitár et al., 2014). In contrast, road verges preserved only a small portion of grassland specialist plant species near loess grasslands in our study area because of frequent mowing and trampling (Deák et al., 2020, 2021b).

The absence of fragmentation effects of the current landscape can also refer to an extinction debt, that is a time-delayed extinction to be realised in the future without further habitat modification after habitat loss in the past (Kuussaari et al., 2009). A weak extinction debt was shown in the sandy steppe study region by Rédei et al. (2014), who found that the richness of sand specialist flora reflected the historical landscape composition in the 18th century, but they did not find any correlation with the present composition. Deák et al. (2021a) also showed for kurgans that the landscape structure of the 1970s best explained the species richness of loess grassland specialists, referring to an unpaid extinction debt. Their results are not in contradiction with our results, as they showed that contemporary connectivity explained specialist richness almost as well as that in the 1970s. It is also possible that the local management regime (e.g., mowing or grazing) not assessed in this study overrode and obscured fragmentation impacts (Gallé et al., 2022).

5. Conclusion

We found that habitat specialist plant species were less dependent on connectivity and habitat area than generalists and exotic species in sand and loess steppe fragments. However, there may be an extinction debt in the effect of fragmentation. Both habitat connectivity and fragment size, two important measures of habitat fragmentation, affected vegetation composition regarding local persistence and dispersal potential-related traits. Based on our analyses, we can predict how shifted species composition would enable communities to respond to further habitat fragmentation if they still own regeneration and resilience potential. We may rely on the persistence of long-lived and clonal specialist and generalist species in fragmented sand and loess steppes, as they showed no response for fragment size and connectivity loss. The regeneration potential of the vegetation following fragmentation can be improved by preserving large fragments and increasing habitat connectivity in parallel. However, given the fact that these also do not buffer against exotic species, active and targeted management of exotic species is also important, especially in times of climate change extremely threatening these habitats.

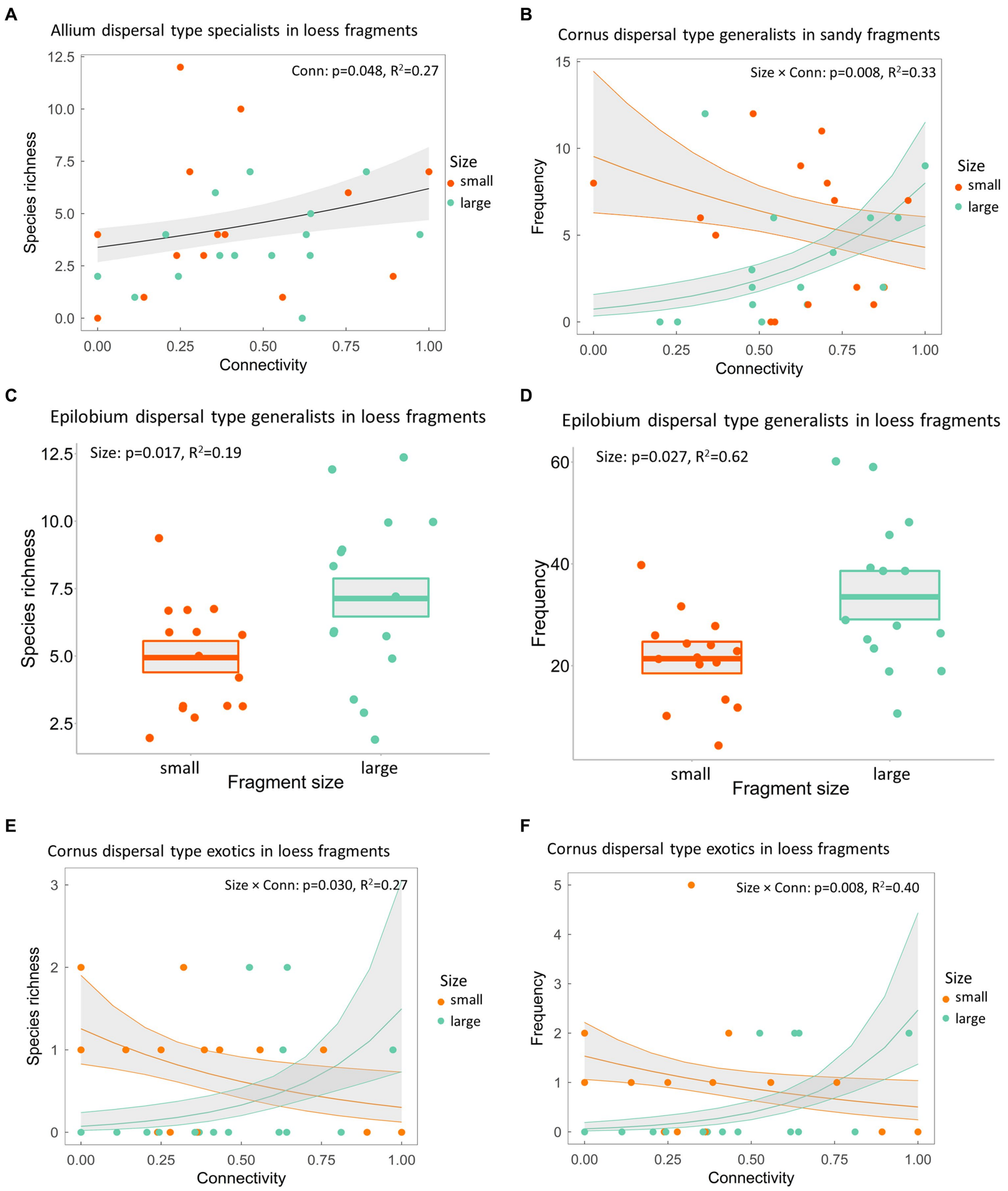


FIGURE 4
(A) Richness of specialist species with Allium-type dispersal strategy in loess steppe fragments, **(B)** frequency of generalist species with Cornus-type dispersal strategy in sandy fragments and **(C)** species richness and **(D)** frequency of generalists with Epilobium-type dispersal strategy, **(E)** species richness and **(F)** frequency of exotic species with Cornus-type dispersal in loess grassland fragments. p-value shows the statistical significance of the explanatory variable(s), R^2 stands for the conditional coefficient of determination of the model, whereas the grey shaded area represent the standard error of the mean.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

PB, RG, and KS designed the study. CT, BD, RG, and DK performed data collection and preparation. KS performed data analyses with substantial input from DK. KS wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Hungarian National Research, Development and Innovation Office (NKFIH KKP 133839).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1155885/full#supplementary-material

References

Amartuvshin, N., Hülber, K., Plutzar, C., and Tserenbaljid, G. (2019). Functional traits but not environmental gradients explain seed weight in Mongolian plant species. *Plant Biol.* 21, 559–562. doi: 10.1111/plb.12938

Auffret, A. G., Kimberley, A., Plue, J., and Waldén, E. (2018). Super-regional land-use change and effects on the grassland specialist flora. *Nat. Commun.* 9:3464. doi: 10.1038/s41467-018-05991-y

Auffret, A. G., Plue, J., and Cousins, S. A. (2015). The spatial and temporal components of functional connectivity in fragmented landscapes. *Ambio* 44, 51–59. doi: 10.1007/s13280-014-0588-6

Bacles, C. F., Lowe, A. J., and Ennos, R. A. (2006). Effective seed dispersal across a fragmented landscape. *Science* 311:628. doi: 10.1126/science.1121543

Bakker, J. P., Poschlod, P., Strykstra, R. J., Bekker, R. M., and Thompson, K. (1996). Seed banks and seed dispersal: important topics in restoration ecology. *Acta Bot. Neerl.* 45, 461–490. doi: 10.1111/j.1438-8677.1996.tb00806.x

Barton, K. (2022). MuMIn: Multi-model inference. R package version 1.1. Available at: https://cran.r-project.org/package=MuMIn (Accessed 15 January 2023).

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2021). lme4: Linear Mixed-Effects Models using "Eigen" and S4. ver 1.1-27.1 https://cran.r-project.org/web/packages/lme4/index.html (Accessed 20 January 2022).

Biró, M., Révész, A., Molnár, Z., Horváth, F., and Czúcz, B. (2008). Regional habitat pattern of the Duna-Tisza köze in Hungary II. The sand, the steppe and the riverine vegetation: degraded and ruined habitats. *Acta Bot. Hung.* 50, 19–60. doi: 10.1556/ABot.50.2008.1-2.2

Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., et al. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R. I. Dent. J.* 9, 378–400. doi: 10.3929/ethz-b-000240890

Brückmann, S. V., Krauss, J., and Steffan-Dewenter, I. (2010). Butterfly and plant specialists suffer from reduced connectivity in fragmented landscapes. *J. Appl. Ecol.* 47, 799–809. doi: 10.1111/j.1365-2664.2010.01828.x

Csecserits, A., Botta-Dukát, Z., Kröel-Dulay, G., Lhotsky, B., Ónodi, G., Rédei, T., et al. (2016). Tree plantations are hot-spots of plant invasion in a landscape with heterogeneous land-use. *Agric. Ecosyst. Environ.* 226, 88–98. doi: 10.1016/j.agee.2016.03.024

Csecserits, A., Czúcz, B., Halassy, M., Kröel-Dulay, G., Rédei, T., Szabó, R., et al. (2011). Regeneration of sandy old-fields in the forest steppe region of Hungary. *Plant Biosyst.* 145, 715–729. doi: 10.1080/11263504.2011.601340

Csontos, P., Tamás, J., and Balogh, L. (2003). Thousand seed weight records of species from the flora of Hungary, I. Monocotyledonopsida. *Stud. Bot. Hung.* 34, 121–126.

Csontos, P., Tamás, J., and Balogh, L. (2007). Thousand seed weight records of species from the flora of Hungary, II. Dicotyledonopsida. *Stud. Bot. Hung.* 38, 179–189.

Deák, B., Bede, Á., Rádai, Z., Tóthmérész, B., Török, P., Torma, A., et al. (2021a). Different extinction debts among plants and arthropods after loss of grassland amount and connectivity. *Biol. Conserv.* 264:109372. doi: 10.1016/j.biocon.2021.109372

Deák, B., Rádai, Z., Bátori, Z., Kelemen, A., Lukács, K., Kiss, R., et al. (2021b). Ancient burial mounds provide safe havens for grassland specialist plants in transformed landscapes—a trait-based analysis. *Front. Ecol. Evol.* 9:619812. doi: 10.3389/fevo.2021.619812

Deák, B., Rádai, Z., Lukács, K., Kelemen, A., Kiss, R., Bátori, Z., et al. (2020). Fragmented dry grasslands preserve unique components of plant species and phylogenetic diversity in agricultural landscapes. *Biodivers. Conserv.* 29, 4091–4110. doi: 10.1007/s10531-020-02066-7

Deák, B., Tóthmérész, B., Valkó, O., Sudnik-Wójcikowska, B., Bragina, T. M., Moysiyenko, I. I., et al. (2016). Cultural monuments and nature conservation: the role of kurgans in maintaining steppe vegetation. *Biodivers. Conserv.* 25, 2473–2490. doi: 10.1007/s10531-016-1081-2

Deák, B., Valkó, O., Török, P., Kelemen, A., Bede, Á., Csathó, A. I., et al. (2018). Landscape and habitat filters jointly drive richness and abundance of specialist plants in terrestrial habitat islands. *Landsc. Ecol.* 33, 1117–1132. doi: 10.1007/s10980-018-0660-x

Dembicz, I., Moysiyenko, I. I., Kozub, Ł., Dengler, J., Zakharova, M., and Sudnik-Wójcikowska, B. (2021). Steppe islands in a sea of fields: where island biogeography meets the reality of a severely transformed landscape. *J. Veg. Sci.* 32:e12930. doi: 10.1111/jvs.12930

Didham, R. K., Kapos, V., and Ewers, R. M. (2012). Rethinking the conceptual foundations of habitat fragmentation research. *Oikos* 121, 161–170. doi: 10.1111/j.1600-0706.2011.20273.x

Didham, R. K., Tylianakis, J. M., Hutchison, M. A., Ewers, R. M., and Gemmell, N. J. (2005). Are invasive species the drivers of ecological change? Trends Ecol. *Evolution* 20, 470–474. doi: 10.1016/j.tree.2005.07.006

Dupré, C., and Ehrlén, J. (2002). Habitat configuration, species traits and plant distributions. *J. Ecol.* 90, 796–805. doi: 10.1046/j.1365-2745.2002.00717.x

Erdős, L., Ambarlı, D., Anenkhonov, O. A., Bátori, Z., Cserhalmi, D., Kiss, M., et al. (2018a). The edge of two worlds: a new review and synthesis on Eurasian forest-steppes. *Appl. Veg. Sci.* 21, 345–362. doi: 10.1111/avsc.12382

Erdős, L., Kröel-Dulay, G., Bátori, Z., Kovács, B., Németh, C., Kiss, P. J., et al. (2018b). Habitat heterogeneity as a key to high conservation value in forest-grassland mosaics. *Biol. Conserv.* 226, 72–80. doi: 10.1016/j.biocon.2018.07.029

Eriksson, O. (1996). Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. *Oikos* 77, 248–258. doi: 10.2307/3546063

European Commission (2013). Interpretation manual of European Union habitats – EUR28. European Commission. Available at: https://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/Int_Manual_EU28.pdf (Accessed 15 December 2022).

Fekete, G., Molnár, Z., Kun, A., and Botta-Dukát, Z. (2002). On the structure of the Pannonian forest steppe: grasslands on sand. *Acta Zool. Hung.* 48, 137–150.

Fröborg, H., and Eriksson, O. (1997). Local colonization and extinction of field layer plants in a deciduous forest and their dependence upon life history features. *J. Veg. Sci.* 8, 395–400. doi: 10.2307/3237329

Gallé, R., Korányi, D., Tölgyesi, C., Lakatos, T., Marcolin, F., Török, E., et al. (2022). Landscape-scale connectivity and fragment size determine species composition of grassland fragments. *Basic Appl. Ecol.* 65, 39–49. doi: 10.1016/j.baae.2022.10.001

Gaujour, E., Amiaud, B., Mignolet, C., and Plantureux, S. (2012). Factors and processes affecting plant biodiversity in permanent grasslands. A review. *Agron. Sustain. Dev.* 32, 133–160. doi: 10.1007/s13593-011-0015-3

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., and Wiezik, M. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodivers. Conserv.* 22, 2131–2138. doi: 10.1007/s10531-013-0537-x

Hanski, I., and Ovaskainen, O. (2000). The metapopulation capacity of a fragmented landscape. *Nature* 404, 755–758. doi: 10.1038/35008063

Hartig, F. (2022). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R project. R package version 0.4.5. Available at: https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html (Accessed 10 January 2022).

Heinken, T., and Weber, E. (2013). Consequences of habitat fragmentation for plant species: do we know enough? *Perspect. Plant Ecol. Evol. Syst.* 15, 205–216. doi: 10.1016/j.ppees.2013.05.003

Hemrová, L., and Münzbergová, Z. (2015). The effects of plant traits on species' responses to present and historical patch configurations and patch age. *Oikos* 124, 437–445. doi: 10.1111/oik.01130

Honnay, O., and Bossuyt, B. (2005). Prolonged clonal growth: escape route or route to extinction? *Oikos* 108, 427–432. doi: 10.1111/j.0030-1299.2005.13569.x

Honnay, O., Bossuyt, B., Jacquemyn, H., Shimono, A., and Uchiyama, K. (2008). Can a seed bank maintain the genetic variation in the above ground plant population? *Oikos* 117, 1–5. doi: 10.1111/j.2007.0030-1299.16188.x

Isbell, F., Balvanera, P., Mori, A. S., He, J. S., Bullock, J. M., Regmi, G. R., et al. (2022). Expert perspectives on global biodiversity loss and its drivers and impacts on people. *Front. Ecol. Environ.* 21, 94–103. doi: 10.1002/fee.2536

Jackson, S. T., and Sax, D. F. (2010). Balancing biodiversity in a changing environment: extinction debt, immigration credit and species turnover. *Trends Ecol. Evol.* 25, 153–160. doi: 10.1016/j.tree.2009.10.001

Janečková, P., Janeček, S., Klimešová, J., Götzenberger, L., Horník, J., Lepš, J., et al. (2017). The plant functional traits that explain species occurrence across fragmented grasslands differ according to patch management, isolation, and wetness. *Landsc. Ecol.* 32, 791–805. doi: 10.1007/s10980-017-0486-y

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., et al. (2020). TRY plant trait database–enhanced coverage and open access. *Glob. Chang. Biol.* 26, 119–188. doi: 10.1111/gcb.14904

Király, G. (2009). *New Hungarian herbal. The vascular plants of Hungary. Identification keys.* Aggteleki National Park Directorate, Jósvafő, Hungary. 616.

Kleyer, M., Bekker, R. M., Knevel, I. C., Bakker, J. P., Thompson, K., Sonnenschein, M., et al. (2008). The LEDA Traitbase: a database of life-history traits of the northwest European flora. *J. Ecol.* 96, 1266–1274. doi: 10.1111/j.1365-2745.2008.01430.x

Klimešová, J., and de Bello, F. (2009). CLO-PLA: the database of clonal and bud bank traits of central European flora. *J. Veg. Sci.* 20, 511–516. doi: 10.1111/j.1654-1103.2009.01050.x

Kuli-Révész, K., Korányi, D., Lakatos, T., Szabó, Á. R., Batáry, P., and Gallé, R. (2021). Smaller and isolated grassland fragments are exposed to stronger seed and insect predation in habitat edges. *Forests* 12:54. doi: 10.3390/f12010054

Kuussaari, M., Bommarco, R., Heikkinen, R. K., Helm, A., Krauss, J., Lindborg, R., et al. (2009). Extinction debt: a challenge for biodiversity conservation. *Trends Ecol. Evol.* 24, 564–571. doi: 10.1016/j.tree.2009.04.011

Lindborg, R. (2007). Evaluating the distribution of plant life-history traits in relation to current and historical landscape configurations. *J. Ecol.* 95, 555–564. doi: 10.1111/j.1365-2745.2007.01232.x

Lindborg, R., Helm, A., Bommarco, R., Heikkinen, R. K., Kühn, I., Pykälä, J., et al. (2012). Effect of habitat area and isolation on plant trait distribution in European forests and grasslands. *Ecography* 35, 356–363. doi: 10.1111/j.1600-0587.2011.07286.x

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., et al. (2001). Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808. doi: 10.1126/science.1064088

Marini, L., Bruun, H. H., Heikkinen, R. K., Helm, A., Honnay, O., Krauss, J., et al. (2012). Traits related to species persistence and dispersal explain changes in plant communities subjected to habitat loss. *Divers. Distrib.* 18, 898–908. doi: 10.1111/j.1472-4642.2012.00893.x

Maurer, K., Durka, W., and Stöcklin, J. (2003). Frequency of plant species in remnants of calcareous grassland and their dispersal and persistence characteristics. *Basic Appl. Ecol.* 4, 307–316. doi: 10.1078/1439-1791-00162

McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* 21, 178–185. doi: 10.1016/j.tree.2006.02.002

Moilanen, A., and Hanski, I. (2001). On the use of connectivity measures in spatial ecology. *Oikos* 95, 147–151. doi: 10.1034/j.1600-0706.2001.950116.x

Molnár, Z., Biró, M., Bartha, S., and Fekete, G. (2012). "Past trends, present state and future prospects of Hungarian forest-steppes" in *Eurasian steppes. Ecological problems and livelihoods in a changing world*. eds. M. J. A. Werger and M. A. van Staalduinen (Dordrecht, Heidelberg, New York: Springer), 209–252.

Öckinger, E., Schweiger, O., Crist, T. O., Debinski, D. M., Krauss, J., Kuussaari, M., et al. (2010). Life-history traits predict species responses to habitat area and isolation: a cross-continental synthesis. *Ecol. Lett.* 13, 969–979. doi: 10.1111/j.1461-0248.2010.01487.x

Ónodi, G., Kertész, M., Lengyel, A., Pándi, I., Somay, L., Szitár, K., et al. (2021). The effects of woody plant encroachment and wildfire on plant species richness and composition: temporal changes in a forest–steppe mosaic. *Appl. Veg. Sci.* 24:e12546. doi: 10.1111/avsc.12546

Ottaviani, G., Keppel, G., Götzenberger, L., Harrison, S., Opedal, Ø. H., Conti, L., et al. (2020). Linking plant functional ecology to island biogeography. *Trends Plant Sci.* 25, 329–339. doi: 10.1016/j.tplants.2019.12.022

Pandit, S. N., Kolasa, J., and Cottenie, K. (2009). Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. *Ecology* 90, 2253–2262. doi: 10.1890/08-0851.1

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., et al. (2017). Package 'nlme'. Linear and nonlinear mixed effects models, version, 3. Available at: https://cran.r-project.org/web/packages/nlme/nlme.pdf (Accessed 20 January 2022).

QGIS Development Team. (2019). Quantum GIS geographic information system. Open source geospatial foundation project. Available at: http://qgis.osgeo.org (Accessed 1 January 2021).

R Core Team. (2022). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rédei, T., Szitár, K., Czúcz, B., Barabás, S., Lellei-Kovács, E., Pándi, I., et al. (2014). Weak evidence of long-term extinction debt in Pannonian dry sand grasslands. *Agric. Ecosyst. Environ.* 182, 137–143. doi: 10.1016/j.agee.2013.07.016

Rösch, V., Tscharntke, T., Scherber, C., and Batáry, P. (2013). Landscape composition, connectivity and fragment size drive effects of grassland fragmentation on insect communities. *J. Appl. Ecol.* 50, 387–394. doi: 10.1111/1365-2664.12056

Sádlo, J., Chytrý, M., Pergl, J., and Pyšek, P. (2018). Plant dispersal strategies. *Preslia* 90, 1–22. doi: 10.23855/preslia.2018.001

Sonkoly, J., Tóth, E., Balogh, N., Balogh, L., Bartha, D., Bata, K., et al. (2022). PADAPT 1.0–the Pannonian database of plant traits. bioRxiv [Preprint].

Soons, M. B., Messelink, J. H., Jongejans, E., and Heil, G. W. (2005). Habitat fragmentation reduces grassland connectivity for both short-distance and long-distance wind-dispersed forbs. *J. Ecol.* 93, 1214–1225. doi: 10.1111/j.1365-2745.2005.01064.x

Squires, V. R., and Feng, H. (2018). "Brief history of grassland utilization and its significance to humans" in *Grasslands of the world*. eds. V. R. Squires, J. Dengler, L. Hua and H. Feng (Boca Raton, FL: CRC Press), 17–26.

Szitár, K., Ónodi, G., Somay, L., Pándi, I., Kucs, P., and Kröel-Dulay, G. (2014). Recovery of inland sand dune grasslands following the removal of alien pine plantation. *Biol. Conserv.* 171, 52–60. doi: 10.1016/j.biocon.2014.01.021

Tanács, E., Belényesi, M., Lehoczki, R., Pataki, R., Petrik, O., Standovár, T., et al. (2021). Compiling a high-resolution country-level ecosystem map to support environmental policy: methodological challenges and solutions from Hungary. *Geocarto Int.* 37, 8746–8769. doi: 10.1080/10106049.2021.2005158

Taylor, P. D., Fahrig, L., Henein, K., and Merriam, G. (1993). Connectivity is a vital element of landscape structure. *Oikos* 68, 571–573. doi: 10.2307/3544927

Tilman, D., May, R. M., Lehman, C. L., and Nowak, M. A. (1994). Habitat destruction and the extinction debt. *Nature* 371, 65–66. doi: 10.1038/371065a0

Török, P., Miglécz, T., Valkó, O., Tóth, K., Kelemen, A., Albert, Á. J., et al. (2013). New thousand-seed weight records of the Pannonian flora and their application in analysing social behaviour types. *Acta Bot. Hung.* 55, 429–472. doi: 10.1556/abot.55.2013.3-4.17

Török, P., Tóth, E., Tóth, K., Valkó, O., Deák, B., Kelbert, B., et al. (2016). New measurements of thousand-seed weights of species in the Pannonian flora. *Acta Bot. Hung.* 58, 187–198. doi: 10.1556/034.58.2016.1-2.10

Turner, I. M. (1996). Species loss in fragments of tropical rain forest: a review of the evidence. *J. Appl. Ecol.* 33, 200–209. doi: 10.2307/2404743

Uroy, L., Ernoult, A., and Mony, C. (2019). Effect of landscape connectivity on plant communities: a review of response patterns. *Landsc. Ecol.* 34, 203–225. doi: 10.1007/s10980-019-00771-5

Vázquez, D. P., and Simberloff, D. (2002). Ecological specialization and susceptibility to disturbance: conjectures and refutations. *Am. Nat.* 159, 606–623. doi: 10.1086/339991

Vittoz, P., and Engler, R. (2007). Seed dispersal distances: a typology based on dispersal modes and plant traits. *Bot. Helv.* 117, 109–124. doi: 10.1007/s00035-007-0797-8

Wickham, H. (2011). ggplot2. *Wiley Interdiscip. Rev. Comput. Stat.* 3, 180–185. doi: 10.1002/wics.147

Wild, J., Kaplan, Z., Danihelka, J., Petřík, P., Chytrý, M., Novotný, P., et al. (2019). Plant distribution data for the Czech Republic integrated in the Pladias database. *Preslia* 91, 1–24. doi: 10.23855/preslia.2019.001

Wilson, J. B., Peet, R. K., Dengler, J., and Pärtel, M. (2012). Plant species richness: the world records. *J. Veg. Sci.* 23, 796–802. doi: 10.1111/j.1654-1103.2012.01400.x

Yan, Y., Jarvie, S., Liu, Q., and Zhang, Q. (2022). Effects of fragmentation on grassland plant diversity depend on the habitat specialization of species. *Biol. Conserv.* 275:109773. doi: 10.1016/j.biocon.2022.109773

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 25 May 2023
DOI 10.3389/fevo.2023.1148266

EDITED BY
Armin Bischoff,
University of Avignon, France

REVIEWED BY
Paulo A. V. Borges,
University of the Azores, Portugal
Olivier Blight,
University of Avignon, France

*CORRESPONDENCE
Felix Helbing
✉ felix.helbing@uos.de

Received 19 January 2023
ACCEPTED 02 May 2023
PUBLISHED 25 May 2023

CITATION
Helbing F, Fartmann T, Morkel C and Poniatowski D (2023) Rapid response of vascular plants and insects to restoration of montane grasslands.
Front. Ecol. Evol. 11:1148266.
doi: 10.3389/fevo.2023.1148266

Rapid response of vascular plants and insects to restoration of montane grasslands

Felix Helbing[1]*, Thomas Fartmann[1,2], Carsten Morkel[3] and Dominik Poniatowski[1]

[1]Department of Biodiversity and Landscape Ecology, Osnabrück University, Osnabrück, Germany, [2]Institute of Biodiversity and Landscape Ecology (IBL), Münster, Germany, [3]Institute of Applied Entomology, Beverungen, Germany

Introduction: Industrialization has ushered in massive changes in agriculture. Particularly in low mountain ranges, large-scale afforestation with Norway spruce on traditionally managed, semi-natural grasslands has caused a severe decline in biodiversity. Tree removal, hay transfer and resumption of grazing or mowing are typical measures to re-create species-rich grasslands. The aim of this study was to use vascular plants and three insect taxa (leafhoppers, true bugs, and grasshoppers) as bioindicators to evaluate the success of montane grassland restoration on former spruce forests in Central Europe. In addition, we intended to identify the drivers of species richness within the studied grasslands in order to derive suitable recommendations for habitat management.

Methods: We analyzed two different treatments: (i) grazed restoration sites where trees had been cut and species-rich green hay had been applied (N =9) and (ii) target sites with a long continuity of low-intensity grazing (N =9).

Results and Discussion: Our study revealed that all studied taxa responded rapidly to the restoration measures. After a development period of 3 to 5 years, we found no differences in species richness and diversity of leafhoppers, true bugs and grasshoppers (all, target and threatened species). In addition, non-metric multidimensional scaling showed a large overlap in species composition between restoration and target grasslands. Among target and threatened species, vascular plants displayed the same pattern as insects and reached similar values when comparing the two treatments. However, total species richness and diversity of vascular plants were still higher on the target sites and species composition overlapped only partially. Grazing intensity was the predictor with the highest explanatory power in multivariable (Generalized) Linear Mixed-effects Models, being negatively related to species richness of leafhoppers and true bugs. We conclude that the measures implemented were effective in re-establishing target communities of different taxa. The transfer of seed-containing hay enabled or accelerated the development of the vegetation. Insects, on the other hand, were able to recolonize the restored grasslands on their own, given that these sites were embedded in a network of species-rich grasslands. With regard to insects (e.g., leafhoppers and true bugs), it should be ensured that grazing is applied at low intensity.

KEYWORDS

Auchenorrhyncha, green hay transfer, habitat quality, Heteroptera, host plant, nature conservation, Orthoptera, *Picea abies*

Introduction

For centuries, European landscapes were shaped by traditional land-use practices (Plieninger et al., 2006; Eriksson, 2021; Fartmann, 2023). Small-scale low-intensity farming created a wide variety of interconnected habitats, considerably fostering biodiversity (Plieninger et al., 2006; Halada et al., 2011). With the onset of industrialization, however, drastic changes in land use were ushered in throughout Europe, accelerating from the mid-20th century onwards (Fartmann, 2023). A wide range of organizational and technological innovations, such as land consolidation instruments, progressive mechanization and the increasing use of artificial fertilizers, enabled large-scale intensification of agriculture on productive soils (Stoate et al., 2001; Robinson and Sutherland, 2002; Kleijn et al., 2009). By contrast, marginal land that became increasingly unviable for agricultural use was often abandoned or afforested (Henle et al., 2008; Fartmann, 2023). The transformation from traditional to industrial agricultural landscapes is today considered the major driver of the dramatic decrease in farmland biodiversity across Europe (Plieninger et al., 2006; Pereira et al., 2012; Fartmann, 2023).

Temperate, semi-natural grasslands, arising from traditional management, are very rich in plant and animal species and therefore have an outstanding value for nature conservation (Bonari et al., 2017; Feurdean et al., 2018; Fartmann et al., 2021). However, for decades, they have suffered particularly from persistent pressure exerted by land-use change, which has resulted in continuous habitat loss, deterioration and fragmentation (Isselstein et al., 2005; Fartmann et al., 2021; Fartmann, 2023). The contrasting drivers of this development have been unevenly distributed along elevation gradients: the conversion of semi-natural grassland to cropland and the intensification of management have been more pronounced in lowlands, whereas spontaneous (i.e., succession after abandonment) and artificial afforestation on former grasslands have especially taken place at higher elevations (Mottet et al., 2006; Fartmann, 2023).

In Central European low mountain ranges, montane grasslands in particular have been widely planted with introduced Norway spruce (*Picea abies*; Hölzel and Tischew, 2019). This has led to rapid changes in habitat conditions. Following site preparation, trees severely alter light and soil conditions as they grow, hampering the development of ground vegetation (Strand et al., 2021). Grassland plant and animal species not only lose habitat but are also hindered in dispersal between remaining habitat patches (Poniatowski et al., 2016). Consequently, the conversion of grasslands to shady high forests, primarily intended to produce firewood and timber, has caused a strong decline in montane species, and semi-natural montane grasslands are seriously threatened today (Finck et al., 2017; Hölzel and Tischew, 2019).

Besides preserving and improving extant fragments of semi-natural montane grasslands, habitat restoration is a key component of nature conservation for increasing biodiversity. In the case of spruce forests, restoration measures include clear-cutting and subsequent tillage. However, the soil seed bank is often depleted after decades of forest use and spontaneous recolonization by dispersing propagules is limited for many plant species (Bakker et al., 2002; Kiehl et al., 2010). Target species are therefore actively introduced by transferring green hay or brush-harvested seeds from nearby species-rich donor grasslands to restoration sites (Kiehl et al., 2010; Durbecq et al., 2022).

However, the success of implemented measures varies between restoration projects as it depends on several factors, such as timing, species composition of donor sites, soil conditions and effectiveness of reintroduced habitat management (Kiehl et al., 2010; Wagner et al., 2020). A careful evaluation of habitat restoration is thus essential. Contrary to common practice, evaluation studies should ideally focus not only on plants but also include animals, as otherwise there is a risk of overestimating restoration success (Baur, 2014; McAlpine et al., 2016). Although plant recovery is an important prerequisite for animal recolonization, sites may lack suitable habitat quality in terms of microclimate or vegetation structure (Jones and Davidson, 2016; Helbing et al., 2021). Moreover, dispersal-limited species often fail to reach restoration sites spontaneously, especially when dispersal corridors are limited (Baur, 2014; Poniatowski et al., 2016). This applies in particular to arthropods that are major drivers of ecosystem functioning in semi-natural grasslands (Soliveres et al., 2016; Poniatowski et al., 2018; Neff et al., 2020). Studies evaluating both the flora and arthropod fauna of restored montane grasslands, though, are still rare (but see Kurtogullari et al., 2020; Neff et al., 2020). In addition, research to date has tended to focus on the restoration of montane grassland habitats in open areas (e.g., Lencová and Prach, 2011; Sullivan et al., 2020; Durbecq et al., 2022), whereas restoration projects that involve clearing spruce forests are still understudied (but see Dassonville et al., 2013).

The objective of this multi-taxon study was to use vascular plants and three insect taxa (leafhoppers [Hemiptera: Auchenorrhyncha], true bugs [Hemiptera: Heteroptera] and grasshoppers [Orthoptera]) as bioindicators to evaluate the success of montane grassland restoration on former spruce forests in a Central European low mountain range. We compared species richness, diversity and composition of grazed restoration sites and nutrient-poor pastures with long-term grazing history (target grassland). The studied insect taxa (i) play an important role both as consumers and as an abundant food source for insectivores in grassland ecosystems, (ii) occupy different ecological niches, (iii) respond quickly to habitat changes and (iv) allow standardized sampling (Kőrösi et al., 2012; Helbing et al., 2020; Fartmann et al., 2022c). With a development period of 3 to 5 years after the implemented measures, we studied the short-term effects of restoration.

In particular, we addressed the following questions:

(i) Do environmental conditions in restored grasslands differ from those in target grasslands?
(ii) What are the effects of restoration measures on species richness and diversity of vascular plants and insects (all, target and threatened species), and how do species compositions differ between both types of studied grasslands?
(iii) Which environmental parameters explain species richness of vascular plants and insects within the studied grasslands, and, on the basis of the results, which recommendations for further habitat management can be given?

Materials and methods

Study area

The study area is located in the Rothaar Mountains, a low mountain range at the northwestern edge of the German uplands (51°11′17 N, 8°32′25 E; Figure 1). It covers an elevation range from

450 to 842 m a.s.l. and is characterized by a cool (mean annual temperature: 5°C) and humid (mean annual precipitation: 1,450 mm) montane climate with snowy winters (mean snow-cover duration: 100 days/year; Fartmann et al., 2022b). Oligotrophic cambisols on acidic bedrock are the prevalent soils (Geologisches Landesamt Nordrhein-Westfalen (NRW), 1998). Interspersed with grasslands, the landscape is dominated by Norway spruce forests (*Picea abies*), which are non-native to the study region. Although agriculture has been partially intensified here as well, montane grasslands that are grazed or mown at low intensity are still widespread. These comprise mountain hay meadows (order: *Arrhenatheretalia*, alliance: *Polygono-Trisetion*) and species-rich *Nardus* grasslands (order: *Nardetalia strictae*, alliance: *Violion caninae*), both of which are protected under the EU Habitats Directive (92/43/EEC, habitat types 6520 and 6230).

Within the study area, a large-scale EU LIFE project ("Mountain hay meadows near Winterberg") was carried out from 2011 to 2016. It aimed to restore, improve and protect mountain hay meadows and species-rich *Nardus* grasslands. The project measures included the conversion of spruce forests into species-rich grasslands (Schulte, 2017). After the conduction of the restoration measures, the grasslands have been either grazed or mown at low intensity. In this study, we only considered grazed grasslands that were restored on former spruce forests.

Restoration methods

The restoration measures were conducted 3 to 5 years prior to our study (i.e., between 2012 and 2014) and are described in detail in Schulte (2017). Overall, the restoration comprised four steps: (i) deforestation of spruce forests, (ii) removal of remaining branches, (iii) forestry mulching, and (iv) transfer of autochthonous green hay from adjacent donor sites. Only in exceptional cases was the soil additionally milled and harrowed. Long-existing *Nardus* grasslands were rarely accessible by heavy machinery due to topographic and edaphic constraints. Thus, oligotrophic hay meadows with a high number of typical *Nardus* grassland species were mostly chosen as donor sites. For the green hay transfer, these were mown once in early to mid-August of the respective year with a rotary disc mower. The hay was then transported and spread onto the recipient sites using a forage wagon (application rate of 1:1). Only on steeper slopes was spreading by hand necessary.

Study sites and plots

The effectiveness of the restoration measures was evaluated by a comparative approach. We analyzed two treatments:

(i) Montane grasslands where restoration measures described above were carried out and which have since been managed as rough pastures (restored grassland, *N* = 9, mean size: 0.66 ± 0.11 ha, Figures 2A,B).

(ii) Species-rich pastures with a long continuity of low-intensity grazing. These include *Nardus* grasslands and closely related oligotrophic grassland communities. The pastures are characterized by a high phytodiversity and represent the target state of the restoration of grazed grasslands in the study area (target grassland, *N* = 9, mean size: 1.69 ± 0.39 ha, Figures 2C,D).

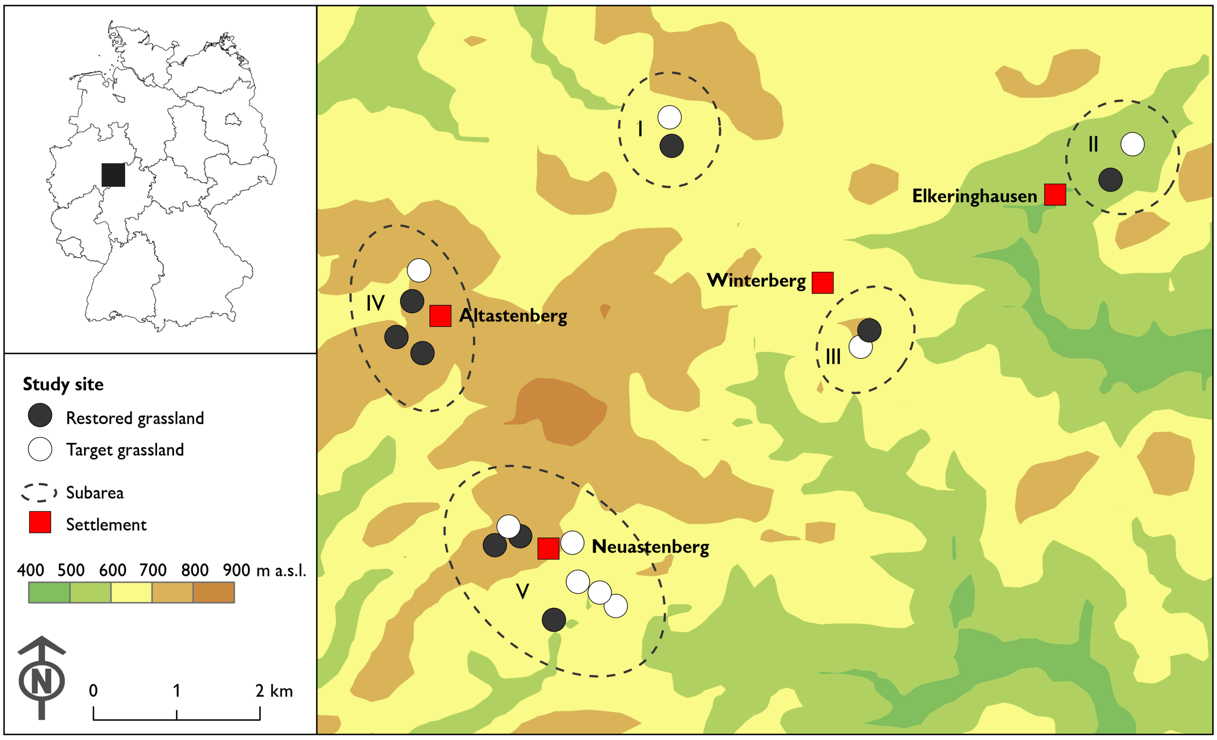


FIGURE 1
Location of the study area and study sites in Germany.

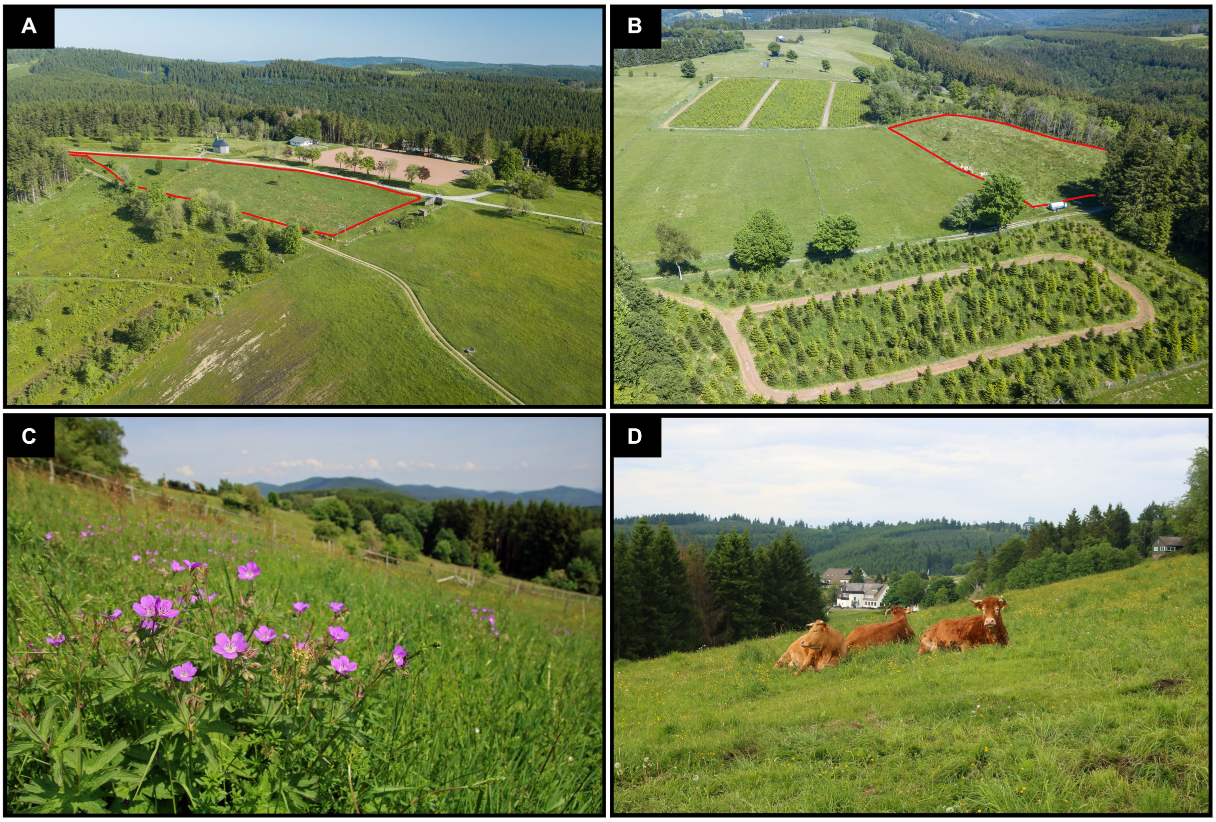


FIGURE 2
Impressions of the study area: **(A,B)** aerial views with restored grasslands framed in red (photos: F. Helbing), **(C)** species-rich pasture with long-term grazing history inhabited by *Geranium sylvaticum* (photo D. Poniatowski) and **(D)** long-term pasture grazed by red cattle (photo D. Poniatowski).

The majority of the sites were grazed by cattle (61%), followed by horses (28%) and goats (11%). In the center of each site, we established a rectangular plot with a total size of 500 m^2 and a distance of at least 10 m to the site boundary (to avoid edge effects; *cf.* Kurtogullari et al., 2020). The plots were marked with magnets and tent pegs, which were placed in the ground together. With the help of a magnetic detector, the locations were accurately retrieved during subsequent visits. In order to address potential issues with spatial autocorrelation, the study area was divided into five subareas, each comprising at least one restored grassland site (Figure 1, see also the "Statistical analysis" section: LMM and GLMM with random effects). We attempted to balance the number of sites of the two treatments within each subarea. However, due to the limited availability of suitable pastures, this was not feasible in subarea IV. We therefore increased the number of studied target grasslands in the adjacent subarea V.

Species sampling and classification

We sampled vascular plants and insects in 2017. Insect sampling was always conducted under favorable weather conditions (dry and sunny days with temperatures > 15°C). Depending on the phenology of each species group, the period and frequency of the surveys varied. In order to evaluate the effects of grassland restoration, we classified three groups of vascular plants and insects: all, target and threatened species. Criteria for defining target species were determined individually for each species group and are described below. If available, we obtained the threat status of the species from the latest red data books of the federal state North Rhine-Westphalia (vascular plants, grasshoppers), otherwise red data books of Germany (leafhoppers, true bugs) were used. Hereinafter, species that are listed as "critically endangered," "endangered," "vulnerable," or "near threatened" (red-list categories: 1, 2, 3 and V) are termed "threatened species." Literature on species identification, scientific nomenclature and the threat status of the studied taxa is listed in Supplementary material.

Vascular plants

We established a subplot covering 16 m^2 (4 m × 4 m) in the center of each plot to record vascular plant species richness and cover. The main vegetation survey was carried out in early June. In early August, we revisited the subplots to search for late-flowering plants such as eyebright (*Euphrasia* spp.). Species cover was estimated according to the Wilmanns scale (Wilmanns, 1998). For statistical analyses, the categories were translated into percentage-cover values: r = 0.1%, + = 0.5%, 1 = 2.5%, 2 m = 2.5%, 2a = 10%, 2b = 20.5%, 3 = 38%, 4 = 63%, and 5 = 88% (Dierschke, 1994).

In addition to the quantitative data gathered within the subplots, we searched the 500 m^2 plots in their entirety for additional plant species during both surveys. From the resulting species list, we determined the host plants of sampled leafhoppers (Nickel and

Remane, 2002; Nickel, 2003) and true bugs (Wachmann et al., 2004, 2006, 2007, 2008) and used their counts as explanatory variables (see the "Statistical analysis" section). Both the donor and target sites were characterized by plant species typical of *Nardus* grasslands as well as mountain hay meadows. Thus, we defined all characteristic species of the phytosociological class *Nardo-Callunetea*, the alliance *Polygono-Trisetion* and their respective subunits as target species (Dierschke, 1997; Oberdorfer, 2001; Peppler-Lisbach and Petersen, 2001; Supplementary Table 1).

Leafhoppers and true bugs

Leafhoppers and true bugs were surveyed together three times in early June, mid-July and early September. We combined a sweep net (30 cm diameter) and a G-Vac suction apparatus (Stihl SH 56, Waiblingen, Germany; 12.5 cm diameter suction tube; 710 m^3h^{-1} air flow rate) with a fine gauze collection bag (300 µm mesh size) on the inner side of the inlet nozzle to cover all available microhabitats and sample the complete range of herb- and ground-dwelling species (*cf.* Stewart, 2002; Helbing et al., 2020). We walked in loops over the whole plot and randomly performed 50 sweep net strokes and 50 suction samples. The latter was done by holding the nozzle onto the ground for 10 s (*cf.* Helbing et al., 2021). The catches were killed with ethyl acetate and identified in the laboratory using a digital microscope (leafhoppers: Keyence VHX-900F, Osaka, Japan) or binoculars (true bugs: Helmut Hund 7–90×, Wetzlar, Germany). Data from both sampling techniques were pooled for statistical analyses. For some genera, identification of females at the species level is not possible (e.g., *Aphrodes*, *Psammotettix*, *Ribautodelphax*). These females were identified at the genus level or, if present, assigned to the corresponding males. In the case of more than one species of a genus, the number of females was assumed to correspond to the number of males (*cf.* Helbing et al., 2021). We defined typical inhabitants of semi-natural grasslands as target species. For leafhoppers, we listed all sampled grassland species that avoid intensive to very intensive management, based on Nickel and Achtziger (1999). From this list, we then removed species that occur exclusively on unmanaged or wet grasslands, as well as pioneer species (Nickel and Achtziger, 1999; Nickel, 2003; Supplementary Table 2). True bug target species were classified according to Wachmann et al. (2004, 2006, 2007, 2008). We included all species that primarily occur on grasslands with a low management intensity except for typical inhabitants of fringe and wetland habitats as well as pioneer species (Supplementary Table 3).

Grasshoppers

Grasshopper sampling took place once per plot in early August. We used a box quadrat ($1.41 \times 1.41\,m \triangleq 2.0\,m^2$), which is a well-approved and accurate tool for surveying species richness and abundance of grasshoppers (Gardiner and Hill, 2006). In each plot, we randomly dropped the box quadrat at 10 different points, covering a total area of 20 m^2 (*cf.* Fartmann et al., 2022a). Species were identified in the field and released afterwards. As with leafhoppers and true bugs, we intended to classify grasshopper species typical of semi-natural grasslands as target species. Poniatowski et al. (2020) presented a species farmland index (SFI) for German grasshopper species that measures the average availability of high nature value farmland (HNV) within a species' range. HNV is a term used to describe biodiversity-rich farming systems, usually resulting from low-intensity traditional land-use practices (Lomba et al., 2014). Habitat specialists that strongly depend on open, semi-natural habitats receive high SFI values, whereas values for habitat generalists are low. In this study, we classified all species with an SFI value of at least 17.0 as target species (Supplementary Table 4).

Environmental conditions

Local climate

We determined the mean elevation (m a.s.l.) of the study sites from topographic maps using the geographical information system ArcGIS 10.6. Moreover, long-term averages of annual temperature (°C) and precipitation (mm; period 1981–2010) were derived from grid maps with a spatial resolution of 1 km^2 (German Meteorological Service, 2022). If sites covered more than one grid cell, we calculated mean values.

During fieldwork, we measured aspect and slope of the plots. In combination with the latitude of the study area (51°), these data were used to calculate the potential amount of direct incident radiation (in MJ cm^{-2} $yr.^{-1}$) as an approximate measure of the local climate (McCune and Keon, 2002).

Habitat characteristics

We calculated the size of the study sites (ha) using orthophotos in ArcGIS 10.6. The intensity of grazing was determined on a metric scale. Combining own observations and information provided by farmers, we recorded the composition of grazing livestock and the duration of grazing (with an accuracy of 0.5 months) for each site during the 2017 season. Each type of animal (species and age) was converted into a livestock unit (LSU) using a conversion key for North Rhine-Westphalia (Table 1; LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), 2022). The grazing intensity of a site was calculated as the product of the density of livestock (summed LSU per hectare) and the months of grazing (MoG).

In the course of the main vegetation survey in June 2017, we recorded parameters of horizontal and vertical habitat structures within each subplot. We estimated the cover of grasses, herbs, cryptogams, bare soil and litter with an accuracy of 5%. If values were above 95% or below 5%, we used steps of 2.5% (Helbing et al., 2021). The vegetation height of the field layer was measured with an accuracy of 2.5 cm. To characterize soil conditions, we also calculated Ellenberg's indicator values for vascular plants (Ellenberg et al., 2001; mean values for moisture, nitrogen, and soil reaction).

TABLE 1 Conversion key for the calculation of livestock units per site [LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen), 2022].

Animal type	Livestock unit
Cattle < 6 months of age	0.4
Cattle 6 months–2 years of age	0.6
Cattle > 2 years of age	1
Horse > 6 months of age	1
Goat	0.15

Statistical analysis

Comparison of habitat types

All statistical analyses were performed using R 4.1.2 (R Core Team, 2022). We quantified the taxonomic richness and diversity of vascular plants and insects using Hill numbers, which provide a mathematically unified set of diversity indices in units of species (Hill, 1973; Jost, 2006; Chao et al., 2014). The sensitivity of these indices to species relative abundances is controlled by the order q (Chao et al., 2014). We considered Hill numbers at $q = 0$, which represents species richness, and at $q = 1$, which is equivalent to the exponential of Shannon entropy (hereinafter termed "diversity"; Jost, 2006; Chao et al., 2014). We tested for significant differences in environmental conditions, species richness and diversity between restored and target grasslands. For the former, we used the Mann–Whitney U-test since the majority of environmental variables showed highly skewed distributions and could thus not be analyzed by mixed-effects models (*cf.* Helbing et al., 2021). By contrast, species richness and diversity of vascular plants, leafhoppers, true bugs and grasshoppers (all, target and threatened species) were compared via Linear Mixed-effects Models (LMM) or Generalized Linear Mixed-effects Models (GLMM) with *habitat type* as a nominal predictor and *subarea* ($N = 5$; see the "Study sites and plots" section) as a random intercept (R package lme4; Bates et al., 2022). For normally distributed and log-transformed variables with a normal distribution, we applied LMMs, otherwise we calculated GLMMs with a negative-binomial error structure to account for overdispersion (Richards, 2008).

Species compositions of restored and target grasslands were compared via non-metric multidimensional scaling (NMDS; R package vegan, metaMDS function; Oksanen et al., 2022) with the Bray-Curtis distance as a distance measure and a maximum number of 200 random starts in search of a stable solution. The four studied species groups were analyzed separately. We included only those species in the analyses that were sampled on at least three plots. For each species group, we performed a Permutational Multivariate Analysis of Variance with 999 permutations (PERMANOVA; Bray-Curtis distance; R package vegan, adonis2 function; Oksanen et al., 2022) to test for significant differences between restored and target grasslands.

Effects of environmental conditions on species richness of vascular plants and insects

We fitted (G)LMMs with the same random effects and error structure as described above to analyze the effects of environmental parameters on species richness of vascular plants and insects (target and threatened species). For each of the studied species groups, we included a specific and ecologically reasonable set of variables into the models (e.g., the number of leafhopper host plant species was used only to analyze leafhopper species richness, whereas Ellenberg indicator values characterizing soil conditions were included only in the modeling of vascular plant species richness). A detailed assignment of the parameters to the species groups is given in Table 2. Prior to the analyses, we standardized all fixed effects to achieve comparability of effect sizes (*cf.* Fumy and Fartmann, 2021). Furthermore, we graphically examined all combinations of response and predictor variables before computing the (G)LMMs. If we detected unimodal rather than linear relationships between two variables, we also entered centered and squared values of the predictor into the full model in addition to the untransformed values (*cf.* Kämpfer and Fartmann, 2022). This was the case for the Ellenberg nitrogen value and threatened vascular plant species. To avoid multicollinearity, we also calculated Spearman's rank correlation (r_S) for all combinations of predictor variables (Dormann et al., 2013). In cases where two or more variables were strongly intercorrelated ($|r_S| \geq 0.6$, $p < 0.05$), only one was included in statistical modeling (Supplementary Table 5). We applied model averaging based on an information-theoretic approach for all multivariable analyses (Grueber et al., 2011). In a first step, all possible combinations of environmental parameters were tested, resulting in a set of different candidate models. These candidate models were then ranked based on Akaike's information criterion ($\mathrm{AIC_C}$) using the "dredge" function (R package MuMIn; Bartoń, 2022). Finally, the top-ranked models within $\Delta\mathrm{AIC_C} < 2$ were used for model averaging (Grueber et al., 2011). We evaluated the explanatory power of the models by calculating marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by both fixed and random effects; Nakagawa et al., 2017). The p-values for LMMs were determined with the R package "lmerTest" (Kuznetsova et al., 2022).

Results

Environmental conditions

Restored and target grasslands differed only slightly in environmental conditions (Table 2). Local climate, grazing intensity, host plants of leafhoppers and true bugs as well as most parameters of habitat structure did not differ. Restored grasslands were merely smaller, had a lower cover of herbs and lower Ellenberg indicator values for nitrogen and soil reaction.

Species richness, diversity, and composition of vascular plants and insects

We sampled a total of 267 species in the four species groups (Table 3; Supplementary Tables 1–4). Vascular plants and leafhoppers were the most species rich taxa, followed by true bugs and grasshoppers. On average, 16% of the species per group were classified as threatened (ranging between 8% in true bugs and 29% in grasshoppers) and 34% as target species (ranging between 15% in vascular plants and 58% in leafhoppers). With a frequency of 100% in both studied habitat types, *Agrostis capillaris*, *Festuca rubra* agg., *Luzula campestris*, and *Rumex acetosa* were the most widespread vascular plant species in our study. The most abundant species of leafhoppers were *Deltocephalus pulicaris* ($N_{Individuals} = 4{,}051$; 22%) and *Verdanus abdominalis* ($N_{Individuals} = 3{,}451$; 18%), those of true bugs were *Pachytomella parallela* ($N_{Individuals} = 955$; 46%) and *Nabis flavomarginatus* ($N_{Individuals} = 385$; 19%), and those of grasshoppers were *Chorthippus biguttulus* ($N_{Individuals} = 512$; 45%) and *Pseudochorthippus parallelus* ($N_{Individuals} = 414$; 36%).

When comparing restored and target grasslands, no differences in species richness and diversity of leafhoppers, true bugs and grasshoppers (all, target and threatened species) were found (Figures 3C–H). This was also true for target and threatened species of vascular plants (Figures 3A,B). It was only the total

TABLE 2 Metric environmental parameters [mean±standard error (SE) as well as minimum (Min) and maximum (Max) values] in restored (N =9) and target (N =9) grasslands.

Parameter	Restored grassland		Target grassland		P	Used for (G)LMM analyses of[a]
	Mean±SE	Min–Max	Mean±SE	Min–Max		
Local climate						
Elevation (m a.s.l.)	698 ± 29	(518–775)	660 ± 23	(531–757)	n.s.	P, L, T, G
Average annual temperature (C°)	6.4 ± 0.1	(5.8–7.2)	6.6 ± 0.1	(5.8–7.2)	n.s.	P, L, T, G
Annual precipitation (mm)	1,282 ± 21	(1,147–1,351)	1,261 ± 21	(1,147–1,351)	n.s.	P, L, T, G
Direct incident radiation[b] (MJ cm^{-2} yr.$^{-1}$)	0.78 ± 0.04	(0.63–0.91)	0.84 ± 0.04	(0.61–0.94)	n.s.	P, L, T, G
Habitat characteristics						
Site size (ha)	0.66 ± 0.11	(0.22–1.25)	1.69 ± 0.39	(0.21–3.94)	*	P, L, T, G
Grazing intensity[c] (LSU MoG ha^{-1})	3.9 ± 0.8	(0.6–7.8)	5.3 ± 1.1	(0.6–8.9)	n.s.	P, L, T, G
Habitat structure						
Cover (%)						
Grasses	74.4 ± 4.5	(55–95)	63.9 ± 4.1	(50–90)	n.s.	L, T, G
Herbs	21.4 ± 4.2	(2.5–40)	40.0 ± 3.8	(15–50)	**	L, T, G
Cryptogams	58.3 ± 7.8	(20–90)	34.2 ± 11.9	(2.5–90)	n.s.	L, T, G
Bare ground	2.5 ± 1.2	(0–10)	3.6 ± 1.0	(0–10)	n.s.	L, T, G
Litter	40.0 ± 9.0	(15–85)	35.0 ± 7.8	(5–85)	n.s.	L, T, G
Vegetation height (cm)	14.9 ± 2.1	(8–27)	14.4 ± 1.8	(8–22)	n.s.	L, T, G
No. vascular plant species						
Leafhopper host plants	18.0 ± 1.7	(12–30)	21.6 ± 1.5	(14–29)	n.s.	L
True bug host plants	16.1 ± 1.5	(12–27)	17.2 ± 1.2	(13–23)	n.s.	T
Ellenberg indicator value (unitless)						
Moisture	5.0 ± 0.1	(4.6–5.8)	5.0 ± 0.1	(4.6–5.3)	n.s.	P
Nitrogen	3.8 ± 0.2	(3.1–4.8)	4.5 ± 0.2	(3.4–5.2)	*	P
Soil reaction	4.0 ± 0.2	(3.1–4.9)	4.8 ± 0.1	(4.3–5.3)	*	P

Differences between the two habitat types were analyzed using the Mann–Whitney U-test. Statistical significances are indicated as follows: n.s., not significant, $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$.

[a] P, Plants; L, Leafhoppers; T, True bugs; G, Grasshoppers.

[b] Direct incident radiation was calculated according to McCune and Keon (2002).

[c] LSU, Livestock units; MoG, Months of grazing (accuracy: 0.5 months; for details see the "Habitat characteristics" section).

TABLE 3 Overview of the studied taxa with numbers of species and individuals [total, mean±standard error (SE) as well as minimum (Min) and maximum (Max) values] per taxon (N_{sites} =18).

Taxon		Number of species			Number of individuals		
Scientific	Alias[a]	Total	Mean±SE	Min–Max	Total	Mean±SE	Min–Max
Tracheophyta	Vascular plants	129	30.3 ± 2.2	14–50	–	–	–
Hemiptera: Auchenorrhyncha	Leafhoppers	73	25.9 ± 0.9	19–32	18,723	1,042.1 ± 93.9	378–1,811
Hemiptera: Heteroptera	True bugs	51	11.3 ± 1.0	4–21	2,073	115.8 ± 14.3	38–255
Orthoptera	Grasshoppers	14	4.9 ± 0.3	4–8	1,151	63.6 ± 10.5	10–145

[a] This term is used throughout the paper.

species richness and the total species diversity of vascular plants that were higher in target than in restored grasslands (Figures 3A,B). NMDS revealed similar patterns in terms of species composition. There was a large overlap in leafhoppers ($F_{1,16} = 1.39$, $p = 0.20$) and grasshoppers ($F_{1,16} = 1.13$, $p = 0.34$) between restored and target grasslands (Figures 4B,D). True bugs also exhibited similarity between the two grassland types, but only for the centrally located target grasslands, while other target grasslands were more dispersed ($F_{1,16} = 4.20$, $p < 0.01$; Figure 4C). Vascular plants showed some overlap between restored and target grasslands as well, but overall, they were more segregated from one another ($F_{1,16} = 4.28$, $p < 0.01$; Figure 4A).

Response of vascular plants and insects to environmental conditions

The (G)LMM analyses identified effects of environmental parameters on species richness of vascular plants, leafhoppers and true bugs. The number of vascular plant target species increased with annual precipitation and decreased with Ellenberg nitrogen values (Table 4A; Figures 5A,B). By contrast, the number of threatened vascular plant species was highest at intermediate Ellenberg nitrogen values (Table 4B; Figure 5C). Grazing intensity was the parameter with the highest

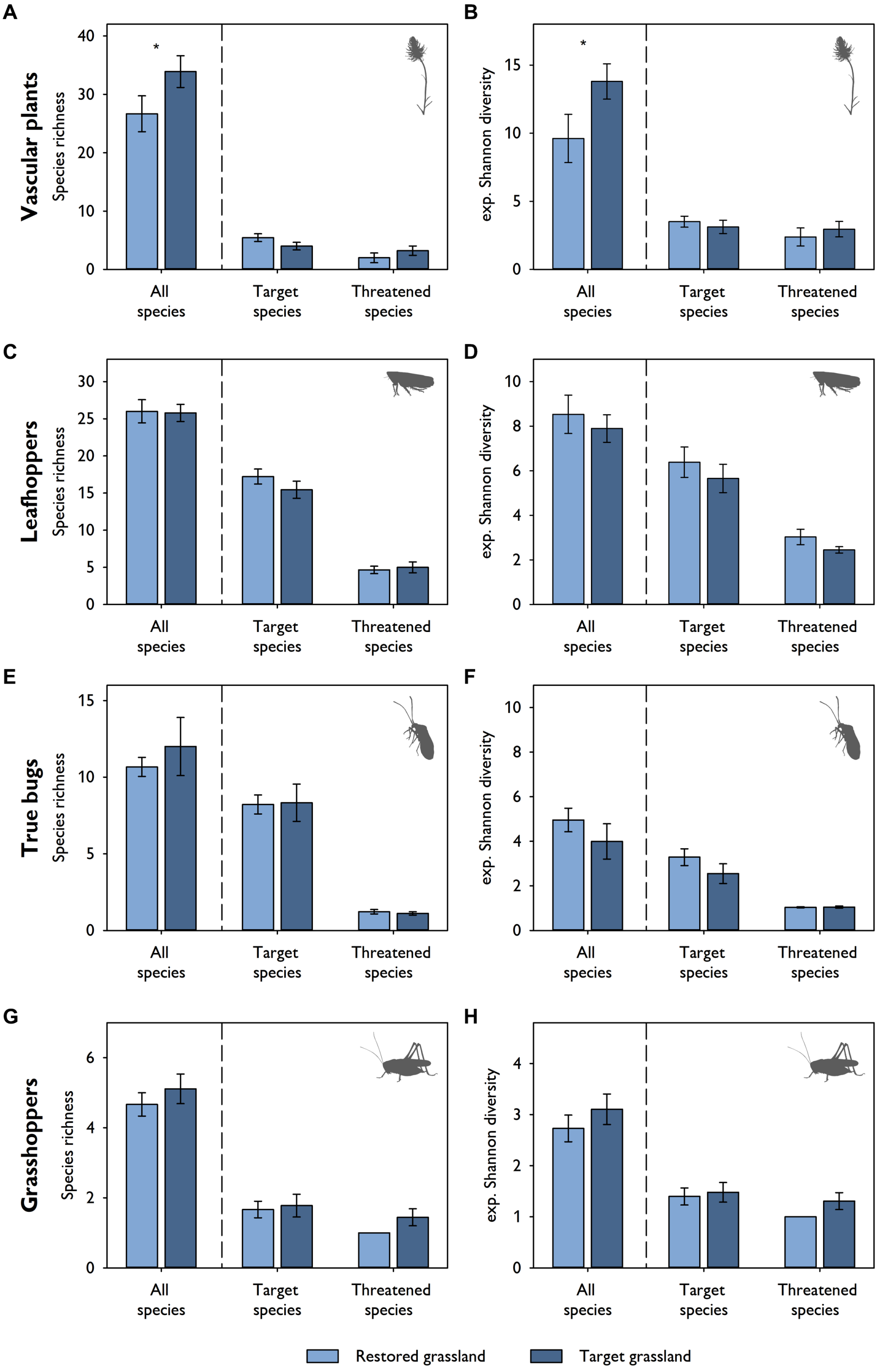


FIGURE 3
Mean values (± standard error) of species richness (Hill numbers of order $q = 0$) and diversity (expressed as exponential Shannon diversity; Hill numbers of order $q = 1$) for all, target and threatened species of **(A,B)** vascular plants, **(C,D)** leafhoppers, **(E,F)** true bugs, and **(G,H)** grasshoppers in restored ($N = 9$) and target grasslands ($N = 9$). Asterisks indicate a significant difference between the two habitat types (tested via mixed-effects models with *subarea* [$N = 5$] as a random intercept; $^*p < 0.05$).

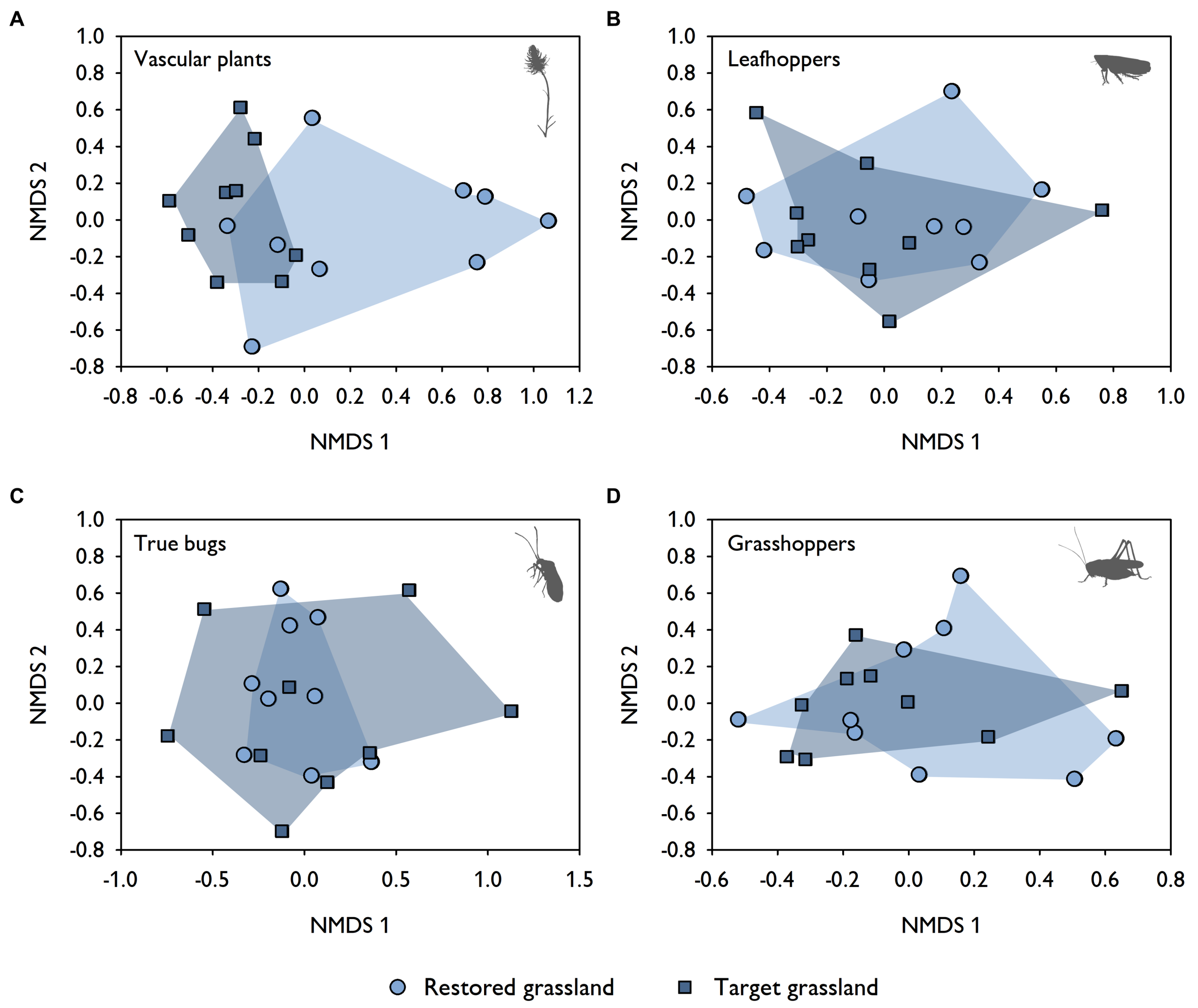


FIGURE 4
NMDS ordinations (Bray–Curtis distance, four dimensions) for assemblages of **(A)** vascular plants (based on 65 species, stress=0.06), **(B)** leafhoppers (based on 47 species and 18,498 individuals, stress=0.10), **(C)** true bugs (based on 22 species and 2,003 individuals, stress=0.09), and **(D)** grasshoppers (based on 8 species and 1,133 individuals, stress=0.05) across restored (*N* =9) and target grasslands (*N* =9).

explanatory power in the multivariable analyses for two of the three insect groups: it negatively affected the species number of leafhoppers (target and threatened species) and true bugs (target species; Tables 4C–E; Figures 5D,E,H). Moreover, direct incident radiation had a positive effect on the number of threatened leafhopper species (Table 4D; Figure 5F) and the number of true bug host plant species was positively related to the number of true bug target species (Table 4E; Figure 5G). For the species richness of grasshoppers (both target and threatened species) and threatened true bugs, there was no relationship with any of the parameters analyzed. The explanatory power of all models was generally high ($R^2_m = 0.30–0.54$; $R^2_c = 0.30–0.63$).

Discussion

Evaluation of restoration measures

Our study revealed that vascular plants and insects (leafhoppers, true bugs, and grasshoppers) responded rapidly to the restoration of species-rich montane grasslands on former spruce forests. After a development period of 3 to 5 years, restored and target grasslands showed a large overlap in the composition of insect species. Furthermore, the studied insect groups neither differed in species richness nor in diversity of all, target and threatened species between the two grassland types. In vascular plants, the same was true for species richness and diversity of target and threatened species. By contrast, total species richness and diversity of vascular plants was still higher in target grasslands and species composition overlapped only partially.

The transfer of seed-containing green hay is an effective method to restore species-rich semi-natural grasslands (Kiehl et al., 2010; Durbecq et al., 2022). Nearby donor sites ensure the preservation of the genetic identity of local plant communities (Albert et al., 2019). In addition, the plant material is usually cut close to the soil surface, enabling the transfer of low-growing plant species as well, and the hay protects seedlings from solar radiation, drought and the competition of ruderal plants (Kiehl et al., 2010; Durbecq et al., 2022). However, a certain proportion of species usually fail to establish on the restoration

TABLE 4 Model-averaging results: influence of environmental parameters (predictor variables) on species richness (Hill numbers of order $q = 0$; target and threatened species) of (A,B) vascular plants, (C,D) leafhoppers, (E,F) true bugs, and (G,H) grasshoppers, analyzed with Linear Mixed-effects Models (LMM) (A,C,D,E) and Generalized Linear Mixed-effects Models (GLMM; negative binomial error structure) (B,F,G,H).

Parameter	Estimate	SE	z/t	*P*	Parameter	Estimate	SE	z	*P*
Vascular plants									
(A) Target species[a] $R^2_{LMMm} = 0.33–0.44$, $R^2_{LMMc} = 0.35–0.44$					(B) Threatened species[b] $R^2_{GLMMm} = 0.30$, $R^2_{GLMMc} = 0.30$				
(Intercept)	1.45	0.12	11.50	***	(Intercept)	1.40	0.34	3.97	***
Ellenberg nitrogen value	−0.30	0.13	2.23	*	Ellenberg nitrogen value (CSV[c])	−1.18	0.50	2.11	*
Annual precipitation	0.26	0.12	2.03	*					
Ellenberg moisture value	0.18	0.11	1.52	n.s.					
Leafhoppers									
(C) Target species[b] $R^2_{LMMm} = 0.51$, $R^2_{LMMc} = 0.51$					(D) Threatened species $R^2_{LMMm} = 0.37–0.54$, $R^2_{LMMc} = 0.45–0.54$				
(Intercept)	15.94	0.51	31.48	***	(Intercept)	4.82	0.33	13.49	***
Grazing intensity	−2.19	0.52	−4.21	***	Grazing intensity	−1.09	0.31	3.17	**
					Direct incident radiation	0.71	0.30	2.16	*
					Herbs	0.65	0.30	1.94	n.s.
True bugs									
(E) Target species $R^2_{LMMm} = 0.31–0.49$, $R^2_{LMMc} = 0.41–0.63$					(F) Threatened species				
(Intercept)	6.34	0.55	10.48	***	n.s.				
No. host plant species	1.10	0.46	2.18	*					
Grazing intensity	−1.08	0.43	2.32	*					
Grasshoppers									
(G) Target species					(H) Threatened species				
n.s.					n.s.				

Model-averaged coefficients (conditional average) were derived from the top-ranked linear models ($\Delta AIC_C < 2$). The variable *subarea* ($N = 5$) was set as a random intercept. All fixed effects were standardized prior to analyses. $R^2_{(G)LMMm}$ = variance explained by fixed effects, $R^2_{(G)LMMc}$ = variance explained by both fixed and random effects (Nakagawa et al., 2017). Statistical significances are indicated as follows: n.s., not significant, $p \geq 0.05$, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

[a] The number of vascular plant target species was log-transformed prior to analysis.

[b] No range for R^2 because model-averaged coefficients were derived from only one model.

[c] CSV, centered and squared values (for details see the "Statistical analysis" section).

sites when green hay is applied (Kiehl et al., 2010; Wagner et al., 2020). As we discuss in the following: (i) limitations inherent to the transfer method and (ii) microsite limitations are the key constrains (Kiehl et al., 2010; Wagner et al., 2020).

The study by Wagner et al. (2020) showed that species with low abundance on a donor site often have a reduced establishment rate on a restoration site. This was probably also the case for some species in the study area. For example, *Nardus stricta*, the eponymous species of *Nardus* grasslands, was absent from most donor sites and was therefore rarely transferred to the restoration sites (Schulte, 2017). Moreover, the timing of hay cutting is always a compromise that may exclude species with an unusual phenology from the transfer of mature seeds (Bakker et al., 2002; Sullivan et al., 2020). In our study, we recorded low establishment rates for several late flowering species such as *Knautia arvensis* and *Sanguisorba officinalis*. This might be explained by the mowing in early to mid-August and hence before seed development had been completed (see also Schulte, 2017; Sullivan et al., 2020).

As with vascular plant and insect species, our study revealed a high degree of similarity between restored and target grasslands in terms of environmental conditions. However, the cover of herbs and Ellenberg indicator values for nitrogen and soil reaction were still lower on the restored grasslands, i.e., on sites that had been covered with spruce forests for decades. Spruce forests are typically characterized by low light availability at the ground and a pronounced layer of needles and other tree components with a limited decomposition rate (Janišová et al., 2007; Fischer and Fischer, 2012). As a consequence, the soil is subject to acidification, a reduced microbial activity and an increased C/N ratio (Fischer and Fischer, 2012; Strand et al., 2021). Additionally, in our case, the restoration sites were only mulched after the trees had been removed, usually without further tillage. Particularly near the mulched tree stumps, this measure produced a layer of wood chips with presumably similar chemical properties as the litter layer (Schulte, 2017). The wood chips may also have limited the direct contact of the seeds with the mineral soil (Schulte, 2017). Nevertheless, according to our results, these sites were suitable for colonization by the majority of target and threatened species adapted to oligotrophic and acidic soils (van Daele et al., 2017; Kurtogullari et al., 2020). Seedling establishment of other grassland species, however, was partially hampered by site characteristics (Roem and Berendse, 2000; Schulte, 2017). This was probably another reason for the differences in species richness, diversity and composition of all vascular plants. There were mainly herbs among the species with low establishment rates, which was also reflected by a generally lower herb cover in restored grasslands. However, the site characteristics in combination with the green hay transfer and subsequent management had the advantage that nitrophilous and ruderal species occurred only rarely on the restored grasslands. In other restoration projects, especially on fertile soils such as on arable land or improved grassland, they may reach high cover and jeopardize the success of the restoration (*cf.* Kiehl et al., 2010; Lencová and Prach, 2011).

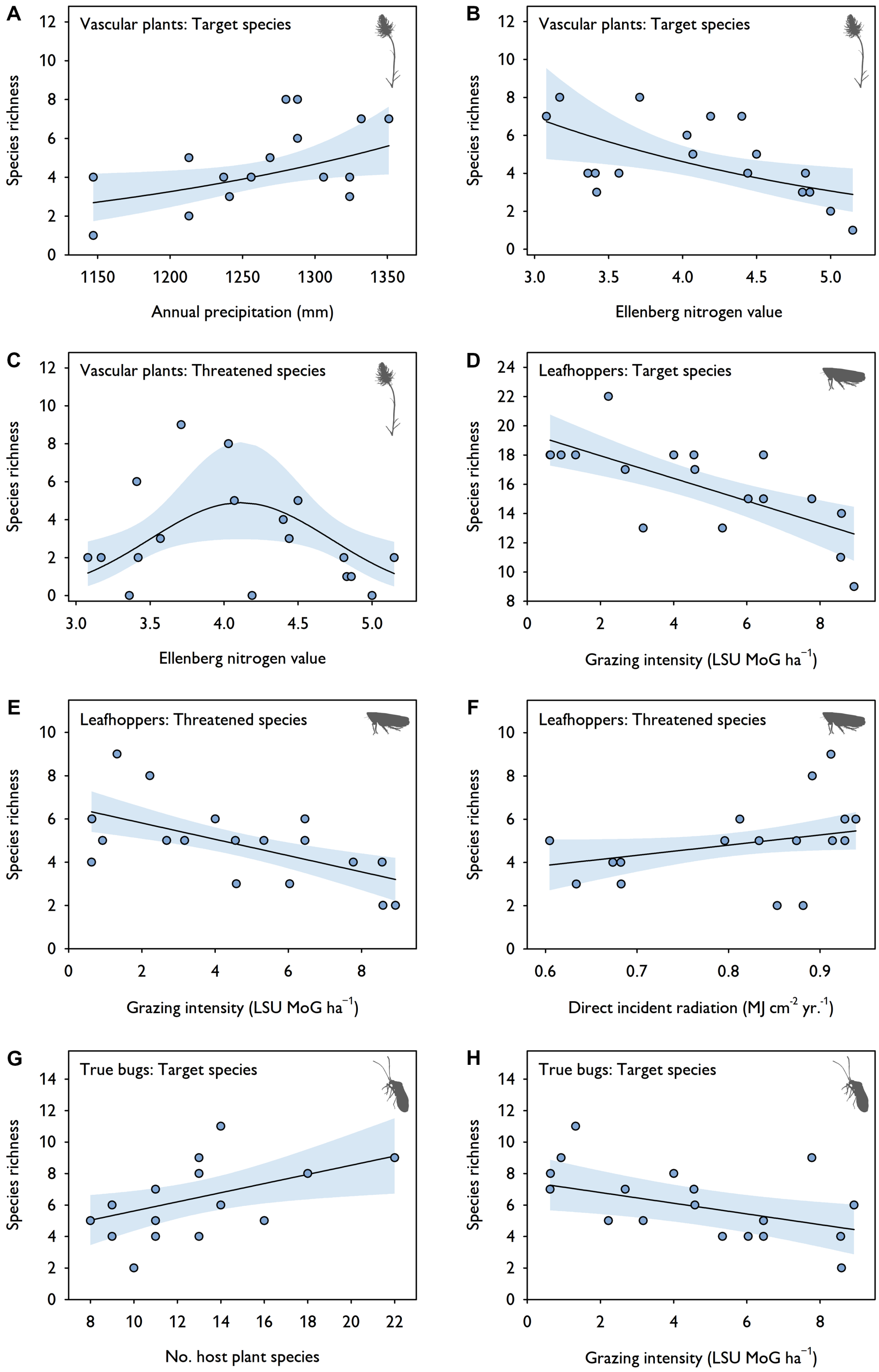


FIGURE 5
Results of mixed-effects model analyses: relationship between significant environmental parameters of the averaged synthesis models and species richness (Hill numbers of order q =0; target and threatened species) of **(A–C)** vascular plants, **(D–F)** leafhoppers, and **(G,H)** true bugs. The regression slopes were fitted using multivariable linear models (see Table 4 for detailed model statistics). No relationships are presented for threatened true bugs and for grasshoppers as the analyses did not reveal any significant parameters. Blue bands indicate 95% confidence intervals.

Similar to plants, the recolonization of a restoration site by insects mainly depends on (i) the availability of species-rich sites with abundant source populations in the surroundings and (ii) a suitable habitat quality (Keene et al., 2020; Helbing et al., 2021). The dispersal ability of the studied insect taxa is largely species-specific. On the one hand, numerous species are known to be highly mobile, being able to move long distances either actively or by passive drift (Reynolds et al., 2017; Florio et al., 2020; Poniatowski et al., 2020). On the other hand, there are many dispersal-limited species that rely on a high degree of habitat connectivity with suitable dispersal corridors to migrate (Nickel, 2003; Littlewood et al., 2009; Poniatowski et al., 2016, 2020). Given that the study area still contains a dense network of species-rich grasslands, we assume that even those species with low mobility were often able to reach restoration sites. One remarkable example is the endangered leafhopper target species *Delphacinus mesomelas*. Although long-winged individuals occasionally occur, the species is usually short-winged. Nevertheless, we sampled it in high abundance on 89% of the restored grasslands, which equals the species' occupation rate on the target grasslands. We suppose that the transfer of the studied insect taxa with the green hay was negligible for species establishment. The number of transferred individuals was very likely rather small, particularly when mown with a disc mower (Stöckli et al., 2021). Moreover, the restoration sites were not vegetated during the conduction of the restoration measures and therefore did not yet provide a suitable habitat for typical insect species of grasslands.

The interaction of trophic resources, spatial structure of the vegetation and microclimatic conditions is often summarized as habitat quality (Mortelliti et al., 2010; Poniatowski et al., 2018; Helbing et al., 2021). Insects that reach a restoration site will only establish if their requirements in habitat quality are fulfilled (*cf.* Helbing et al., 2021; Hussain et al., 2022). As described above, environmental conditions 3 to 5 years after restoration hardly differed between restored and target grasslands. Thus, habitat quality was well suited for successful colonization of the restored sites by grassland insects.

Drivers of species richness

We found only minor differences between restored and target grasslands with regard to species richness of vascular plants and insects as well as environmental conditions. By contrast, the data showed greater variation in species richness (target and threatened species) within all studied grassland sites, and statistical modeling revealed several drivers responsible for this.

The species number of vascular plants was related to the Ellenberg nitrogen value, i.e., to soil fertility. In the case of target species, this relationship was negative. The majority of target species are characteristic of *Nardus* grasslands and are therefore adapted to very oligotrophic conditions (Ellenberg and Leuschner, 2010; van Daele et al., 2017). Only some character species of mountain hay meadows, such as *Crepis mollis*, grow preferentially at more mesotrophic soils. However, these species were hardly present on the study plots. The number of target vascular plant species therefore increased with decreasing soil fertility. For threatened vascular plant species, intermediate Ellenberg nitrogen values resulted in the highest species richness. This unimodal response corresponds with the intermediate productivity hypothesis: it states that only a few species can tolerate the environmental stress at very low productivity and that at the same time a few highly competitive species dominate sites with high productivity (Fraser et al., 2015).

Vascular plant species were also associated with precipitation. The higher the precipitation, the more target species occurred. We assume that an indirect relationship explains this pattern. Precipitation increased with elevation ($r_s = 0.63^{**}$). At higher elevations—i.e. in areas with higher precipitation—the overall intensity of land use is usually lower than at lower elevations (e.g., Strebel and Bühler, 2015; Buzhdygan et al., 2020). Accordingly, we interpret the environmental variable "precipitation" as a proxy for the overall land-use intensity.

Leafhoppers (target and threatened species) and true bugs (target species) were sensitive to grazing intensity. Although the studied gradient was rather small and did not include intensive grazing, it exhibited a negative relationship with species richness. This is typical of both taxa. Numerous studies have shown that species richness and abundance of small insects such as leafhoppers and true bugs often reach the highest values on grasslands with very low land-use intensity or even on abandoned sites (e.g., Dennis et al., 1998; Kruess and Tscharntke, 2002; Biedermann et al., 2005). The reason for this is that species-rich grassland communities characterized by the coexistence of different life strategy types rely on a pronounced field layer with a complex plant architecture (Biedermann et al., 2005; Zurbrügg and Frank, 2006; Helbing et al., 2017, 2021). Each grazing period reduces the height and structural complexity of the vegetation, leading to negative responses in numerous leafhopper and true bug species (Biedermann et al., 2005; Zurbrügg and Frank, 2006). However, very low-intensity grazing or abandonment is favorable only in the short to medium term since succession in the long run results in a lower richness of (host) plant species and an adverse (cool) microclimate (Biedermann et al., 2005). Furthermore, some habitat specialists of both taxa (Nickel, 2003; Wachmann et al., 2004, 2006, 2007, 2008) and other species groups, such as grasshoppers (Fumy et al., 2021), depend on spatial heterogeneity within grasslands, including areas of short turf and bare ground. This is best achieved through a low to intermediate intensity of grazing (van Klink et al., 2015; Fumy et al., 2021).

Threatened leafhopper species were also positively associated with direct incident radiation. Insects are thermophilic organisms, and the local climate of a habitat is an important driver of insect species richness (e.g., Kurtogullari et al., 2020). Helbing et al. (2017) studied leafhopper assemblages on acidic grasslands adjacent to the study area at slightly lower elevations (300–680 m a.s.l.). Numerous of the species detected by the authors were absent in our study. We suggest that climatic limitations may explain these differences. Some species reached their upper range limit in the study area. They probably prefer sites with a warm local climate.

The occurrence of insect herbivores is generally fostered by high plant species richness (Haddad et al., 2001; Joern and Laws, 2013). The majority of true bugs feed on plants, and many species are monophagous or oligophagous (Wachmann et al., 2004, 2006, 2007, 2008). Accordingly, our results revealed a positive relationship between the number of host plant species and the number of target species in true bugs. The same observation was made for leafhoppers in other studies (e.g., Rösch et al., 2013; Helbing et al., 2021). However, although this group of insects is entirely phytophagous and many species exhibit a high degree of host plant specialism (Nickel, 2003; Biedermann et al., 2005), we found no relationship with leafhopper host plants. This is probably a result of the different host plant preferences of leafhoppers and true bugs. For example, a total of 29 sampled leafhopper species (40%) feed on the four vascular plant species that occurred on all study plots (see the "Results" section). In contrast, only 12 true bug species (24%) are known to feed on these widespread plants. We therefore assume that the occurrence of host plants was not a limiting factor for leafhopper species in the study area.

We found no effect of environmental parameters on threatened true bugs or threatened and target grasshoppers. This was likely due to the low number of species in these groups, resulting in limited variance between the sites.

Conclusion

Based on our findings, we conclude that the recovery of species-rich communities of the studied taxa has progressed well in the study area, particularly with respect to insects. The restoration of montane grasslands is thus an important tool for increasing habitat networks and enhancing biodiversity in upland landscapes. This is particularly important in times of global warming since species adapted to cool-humid mountain climates rely on the ability to migrate between habitat patches (Freeman et al., 2018; Stuhldreher and Fartmann, 2018). However, we analyzed only the short-term effects of restoration. The development of the grassland sites should ideally be monitored in the long term. In a positive scenario, species richness and diversity will remain stable or even increase over time. Missing vascular plant species may be reintroduced through selective sowing by conservationists (Wagner et al., 2020). Moreover, species colonization is likely to progress, facilitated by the dense network of grassland patches in the study area (Waldén et al., 2017). On the other hand, target plant species that occur with few individuals on the restoration sites run the risk of not being able to persist in the long term (Kiehl et al., 2010). Moreover, atmospheric nitrogen deposition represents a continuous threat to the studied types of grassland (Bobbink et al., 2010). Insufficient management often results in the dominance of a few grass species such as *Agrostis capillaris* or *Deschampsia flexuosa*, while other species are suppressed (Stevens et al., 2004; Ellenberg and Leuschner, 2010). This also affects insects, as exemplified by true bug host plants. To counteract grass encroachment and in order to promote different insect taxa, including those sensitive to grazing intensity (e.g., leafhoppers and true bugs), lenient grazing with low stocking density is recommended. As an option, annually rotating small areas could be excluded from grazing, as this has been shown to have a positive effect on insect diversity (Kruess and Tscharntke, 2002).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

FH, TF, and DP contributed to the conception and design of the study. FH conducted the field work, identification of leafhopper species in the laboratory and statistical analysis, and wrote the first draft of the manuscript. CM performed the identification of true bug species in the laboratory. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the German Federal Agency for Nature Conservation (Bundesamt für Naturschutz; grant number 3516892017).

Acknowledgments

We sincerely thank Axel M. Schulte (Biologische Station Hochsauerlandkreis e.V.) for his dedicated assistance throughout the research work. Moreover, we are grateful to Raïna Wendland for support during fieldwork, to Herbert Nickel for assistance in the identification of difficult taxa, and to two reviewers for valuable comments on an earlier version of the manuscript. We further acknowledge support by Deutsche Forschungsgemeinschaft (DFG) and Open Access Publishing Fund of Osnabrück University. The restoration measures were co-financed by the EU LIFE project "Mountain hay meadows near Winterberg."

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1148266/full#supplementary-material

References

Albert, Á.-J., Mudrák, O., Jongepierová, I., Fajmon, K., Frei, I., Ševčíková, M., et al. (2019). Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. *Agric. Ecosyst. Environ.* 272, 74–82. doi: 10.1016/j.agee.2018.11.008

Bakker, J. P., Elzinga, J. A., and de Vries, Y. (2002). Effects of long-term cutting in a grassland system: perspectives for restoration of plant communities on nutrient-poor soils. *Appl. Veg. Sci.* 5, 107–120. doi: 10.1111/j.1654-109X.2002.tb00540.x

Bartoń, K. (2022). Multi-model inference (package "MuMIn," version 1.47.1). Available at: https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf (Accessed 21 October 2022).

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2022). Linear mixed-effects models using "Eigen" and S4 (package "lme4," version 1.1–31). Available at: https://cran.r-project.org/web/packages/lme4/lme4.pdf (Accessed 1 November 2022).

Baur, B. (2014). Dispersal-limited species—a challenge for ecological restoration. *Basic Appl. Ecol.* 15, 559–564. doi: 10.1016/j.baae.2014.06.004

Biedermann, R., Achtziger, R., Nickel, H., and Stewart, A. J. A. (2005). Conservation of grassland leafhoppers: a brief review. *J. Insect Conserv.* 9, 229–243. doi: 10.1007/s10841-005-0531-z

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20, 30–59. doi: 10.1890/08-1140.1

Bonari, G., Fajmon, K., Malenovský, I., Zelený, D., Holuša, J., Jongepierová, I., et al. (2017). Management of semi-natural grasslands benefiting both plant and insect diversity: the importance of heterogeneity and tradition. *Agric. Ecosys. Environm.* 246, 243–252. doi: 10.1016/j.agee.2017.06.010

Buzhdygan, O. Y., Tietjen, B., Rudenko, S. S., Nikorych, V. A., and Petermann, J. S. (2020). Direct and indirect effects of land-use intensity on plant communities across elevation in semi-natural grasslands. *PLoS One* 15:e0231122. doi: 10.1371/journal.pone.0231122

Chao, A., Chiu, C.-H., and Jost, L. (2014). Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. *Annu. Rev. Ecol. Evol. Syst.* 45, 297–324. doi: 10.1146/annurev-ecolsys-120213-091540

Dassonville, N., Herremans, J.-P., and Tanghe, M. (2013). Valeur des coupes à blanc de pessières pour la conservation de la biodiversité en Wallonie; restauration de milieux semi-naturels à partir du stock grainier du sol. *Acta Bot. Gallica* 153, 201–218. doi: 10.1080/12538078.2006.10515537

Dennis, P., Young, M. R., and Gordon, I. J. (1998). Distribution and abundance of small insects and arachnids in relation to structural heterogeneity of grazed, indigenous grasslands. *Ecol. Entomol.* 23, 253–264. doi: 10.1046/j.1365-2311.1998.00135.x

Dierschke, H. (1994). *Pflanzensoziologie: Grundlagen und Methoden*. Stuttgart: Eugen Ulmer.

Dierschke, H. (1997). Molinio-Arrhenatheretea (E 1) – Kulturgrasland und verwandte Vegetationstypen, Teil 1: Arrhenatheretalia – Wiesen und Weiden frischer Standorte. *Syn. Pflanzenges. Deutschl.* 3, 1–74.

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., et al. (2013). Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36, 27–46. doi: 10.1111/j.1600-0587.2012.07348.x

Durbecq, A., Rocher, L., Jaunatre, R., Dupré la Tour, A., Buisson, E., and Bischoff, A. (2022). Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover. *Ecol. Eng.* 174:106447. doi: 10.1016/j.ecoleng.2021.106447

Ellenberg, H., and Leuschner, C. (2010). *Vegetation Mitteleuropas mit den Alpen*. Stuttgart: Eugen Ulmer.

Ellenberg, H., Weber, H. E., Düll, R., Wirth, V., Werner, W., and Paulißen, D. (2001). Zeigerwerte von Pflanzen in Mitteleuropa. *Scripta Geobotanica* 18, 1–262.

Eriksson, O. (2021). The importance of traditional agricultural landscapes for preventing species extinctions. *Biodivers. Conserv.* 30, 1341–1357. doi: 10.1007/s10531-021-02145-3

Fartmann, T. (2023). "Grasslands" in *Routledge handbook of insect conservation*. eds. J. Pryke, M. J. Samways, T. New, P. Cardoso and R. Gaigher (London: Routledge)

Fartmann, T., Brüggeshemke, J., Poniatowski, D., and Löffler, F. (2022a). Summer drought affects abundance of grassland grasshoppers differently along an elevation gradient. *Ecol. Entomol.* 47, 778–790. doi: 10.1111/een.13168

Fartmann, T., Drung, M., and Freienstein, M. (2022b). Rejuvenation and restoration measures foster specialised and threatened carabid beetle species in montane heathland ecosystems. *Insect Conserv. Divers.* 15, 348–358. doi: 10.1111/icad.12560

Fartmann, T., Jedicke, E., Stuhldreher, G., and Streitberger, M. (2021). *Insektensterben in Mitteleuropa – Ursachen und Gegenmaßnahmen*. Stuttgart: Eugen Ulmer.

Fartmann, T., Poniatowski, D., and Holtmann, L. (2022c). Effects of land-use and climate change on grasshopper assemblages differ between protected and unprotected grasslands. *Basic Appl. Ecol.* 63, 83–92. doi: 10.1016/j.baae.2022.06.005

Feurdean, A., Ruprecht, E., Molnár, Z., Hutchinson, S. M., and Hickler, T. (2018). Biodiversity-rich European grasslands: ancient, forgotten ecosystems. *Biol. Conserv.* 228, 224–232. doi: 10.1016/j.biocon.2018.09.022

Finck, P., Heinze, S., Raths, U., Riecken, U., and Ssymank, A. (2017). Rote Liste der gefährdeten Biotoptypen Deutschlands. *Naturschutz und Biologische Vielfalt* 156, 1–642.

Fischer, A., and Fischer, H. (2012). "Restoration of temperate forests: an European approach," in *Restoration ecology: The New frontier*, *2nd* Edn., eds AndelJ. van and J. Aronson (Chichester: John Wiley & Sons), 145–160.

Florio, J., Verú, L. M., Dao, A., Yaro, A. S., Diallo, M., Sanogo, Z. L., et al. (2020). Diversity, dynamics, direction, and magnitude of high-altitude migrating insects in the Sahel. *Sci. Rep.* 10:20523. doi: 10.1038/s41598-020-77196-7

Fraser, L. H., Pither, J., Jentsch, A., Sternberg, M., Zobel, M., Askarizadeh, D., et al. (2015). Worldwide evidence of a unimodal relationship between productivity and plant species richness. *Science* 349, 302–305. doi: 10.1126/science.aab3916

Freeman, B. G., Lee-Yaw, J. A., Sunday, J. M., and Hargreaves, A. L. (2018). Expanding, shifting and shrinking: the impact of global warming on species' elevational distributions. *Glob. Ecol. Biogeogr.* 27, 1268–1276. doi: 10.1111/geb.12774

Fumy, F., and Fartmann, T. (2021). Climate and land-use change drive habitat loss in a mountain bird species. *Int J Avian Sci* 163, 1189–1206. doi: 10.1111/ibi.12954

Fumy, F., Kämpfer, S., and Fartmann, T. (2021). Land-use intensity determines grassland Orthoptera assemblage composition across a moisture gradient. *Agric. Ecosyst. Environ.* 315:107424. doi: 10.1016/j.agee.2021.107424

Gardiner, T., and Hill, J. (2006). A comparison of three sampling techniques used to estimate population density and assemblage diversity of Orthoptera. *J. Orthop. Res.* 15, 45–51. doi: 10.1665/1082-6467(2006)15[45:ACOTST]2.0.CO;2

Geologisches Landesamt Nordrhein-Westfalen (NRW). (1998). *Geologische Karte von Nordrhein-Westfalen 1: 100 000, Blatt C 4714 Arnsberg*. Krefeld, Germany: Geologisches Landesamt Nordrhein-Westfalen.

German Meteorological Service. (2022). Vieljährige Mittelwerte. Available at: https://www.dwd.de/DE/leistungen/klimadatendeutschland/vielj_mittelwerte.html (Accessed 17 January 2022).

Grueber, C. E., Nakagawa, S., Laws, R. J., and Jamieson, I. G. (2011). Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* 24, 699–711. doi: 10.1111/j.1420-9101.2010.02210.x

Haddad, N. M., Tilman, D., Haarstad, J., Ritchie, M., and Knops, J. M. H. (2001). Contrasting effects of plant richness and composition on insect communities: a field experiment. *Am. Nat.* 158, 17–35. doi: 10.1086/320866

Halada, L., Evans, D., Romão, C., and Petersen, J.-E. (2011). Which habitats of European importance depend on agricultural practices? *Biodivers. Conserv.* 20, 2365–2378. doi: 10.1007/s10531-011-9989-z

Helbing, F., Fartmann, T., Löffler, F., and Poniatowski, D. (2017). Effects of local climate, landscape structure and habitat quality on leafhopper assemblages of acidic grasslands. *Agric. Ecosyst. Environ.* 246, 94–101. doi: 10.1016/j.agee.2017.05.024

Helbing, F., Fartmann, T., and Poniatowski, D. (2020). Suction samplers are a valuable tool to sample arthropod assemblages for conservation translocation. *Entomol. Exp. Appl.* 168, 688–694. doi: 10.1111/eea.12952

Helbing, F., Fartmann, T., and Poniatowski, D. (2021). Restoration measures foster biodiversity of important primary consumers within calcareous grasslands. *Biol. Conserv.* 256:109058. doi: 10.1016/j.biocon.2021.109058

Henle, K., Alard, D., Clitherow, J., Cobb, P., Firbank, L., Kull, T., et al. (2008). Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe – a review. *Agric. Ecosyst. Environ.* 124, 60–71. doi: 10.1016/j.agee.2007.09.005

Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. *Ecology* 54, 427–432. doi: 10.2307/1934352

Hölzel, N., and Tischew, S. (2019). "Zwergstrauchheiden und bodensaure Magerrasen" in *Renaturierungsökologie*. eds. J. Kollmann, A. Kirmer, S. Tischew, N. Hölzel and K. Kiehl (Berlin: Springer Spektrum), 289–310.

Hussain, R. I., Brandl, M., Maas, B., Krautzer, B., Frank, T., and Moser, D. (2022). Establishing new grasslands on crop fields: short-term development of plant and arthropod communities. *Restor. Ecol.* 30:e13641. doi: 10.1111/rec.13641

Isselstein, J., Jeangros, B., and Vilem, P. (2005). Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe – a review. *Agron. Res.* 3, 139–151.

Janišová, M., Hrivnák, R., Gömöry, D., Ujházy, K., Valachovič, M., Gömöryová, E., et al. (2007). Changes in understorey vegetation after Norway spruce colonization of an abandoned grassland. *Ann. Bot. Fennici* 44, 256–266.

Joern, A., and Laws, A. N. (2013). Ecological mechanisms underlying arthropod species diversity in grasslands. *Annu. Rev. Entomol.* 58, 19–36. doi: 10.1146/annurev-ento-120811-153540

Jones, M. E., and Davidson, N. (2016). Applying an animal-centric approach to improve ecological restoration. *Restor. Ecol.* 24, 836–842. doi: 10.1111/rec.12447

Jost, L. (2006). Entropy and diversity. *Oikos* 113, 363–375. doi: 10.1111/j.2006.0030-1299.14714.x

Kämpfer, S., and Fartmann, T. (2022). Natural coastal dunes on Wadden Sea islands as a refuge for an endangered wader species. *J. Coast. Conserv.* 26:53. doi: 10.1007/s11852-022-00897-w

Keene, K., Malmstrom, C. M., Alexander, H. M., Wayadande, A., and Denning, K. R. (2020). Low conservatism of leafhopper communities in remnant and reconstructed prairie sites in a working agroecological landscape. *J. Insect Conserv.* 24, 35–48. doi: 10.1007/s10841-019-00198-y

Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L., and Hölzel, N. (2010). Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in central and northwestern Europe. *Basic Appl. Ecol.* 11, 285–299. doi: 10.1016/j.baae.2009.12.004

Kleijn, D., Kohler, F., Baldi, A., Batary, P., Concepcion, E., Clough, Y., et al. (2009). On the relationship between farmland biodiversity and land-use intensity in Europe. *Proc. Biol. Sci.* 276, 903–909. doi: 10.1098/rspb.2008.1509

Kőrösi, A., Batáry, P., Orosz, A., Rédei, D., and Báldi, A. (2012). Effects of grazing, vegetation structure and landscape complexity on grassland leafhoppers (Hemiptera: Auchenorrhyncha) and true bugs (Hemiptera: Heteroptera) in Hungary. *Insect Conserv. Divers.* 5, 57–66. doi: 10.1111/j.1752-4598.2011.00153.x

Kruess, A., and Tscharntke, T. (2002). Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biol. Conserv.* 106, 293–302. doi: 10.1016/S0006-3207(01)00255-5

Kurtogullari, Y., Rieder, N. S., Arlettaz, R., and Humbert, J.-Y. (2020). Conservation and restoration of *Nardus* grasslands in the Swiss northern Alps. *Appl. Veg. Sci.* 23, 26–38. doi: 10.1111/avsc.12462

Kuznetsova, A., Brockhoff, P. B., Christensen, R. H. B., and Jensen, S. P. (2022). Tests in linear mixed effects models (package "lmerTest," version 3.1–3). Available at: https://cran.r-project.org/web/packages/lmerTest/lmerTest.pdf (Accessed 22 October 2022).

LANUV (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen) (2022). Umrechnungsschlüssel zur Ermittlung des zulässigen Viehbesatzes. Available at: http://vns.naturschutzinformationen.nrw.de/vns/de/fachinfo/rahmenrichtlinie/umrechnungsschluessel (Accessed 16 May 2022).

Lencová, K., and Prach, K. (2011). Restoration of hay meadows on ex-arable land: commercial seed mixtures vs. spontaneous succession. *Grass Forage Sci.* 66, 265–271. doi: 10.1111/j.1365-2494.2011.00786.x

Littlewood, N. A., Pakeman, R. J., and Woodin, S. J. (2009). Isolation of habitat patches limits colonisation by moorland Hemiptera. *J. Insect Conserv.* 13, 29–36. doi: 10.1007/s10841-007-9114-5

Lomba, A., Guerra, C., Alonso, J., Honrado, J. P., Jongman, R., and McCracken, D. (2014). Mapping and monitoring high nature value farmlands: challenges in European landscapes. *J. Environ. Manage.* 143, 140–150. doi: 10.1016/j.jenvman.2014.04.029

McAlpine, C., Catterall, C. P., Mac Nally, R., Lindenmayer, D., Reid, J. L., Holl, K. D., et al. (2016). Integrating plant- and animal-based perspectives for more effective restoration of biodiversity. *Front. Ecol. Environ.* 14, 37–45. doi: 10.1002/16-0108.1

McCune, B., and Keon, D. (2002). Equations for potential annual direct incident radiation and heat load. *J. Veg. Sci.* 13, 603–606. doi: 10.1111/j.1654-1103.2002.tb02087.x

Mortelliti, A., Amori, G., and Boitani, L. (2010). The role of habitat quality in fragmented landscapes: a conceptual overview and prospectus for future research. *Oecologia* 163, 535–547. doi: 10.1007/s00442-010-1623-3

Mottet, A., Ladet, S., Coqué, N., and Gibon, A. (2006). Agricultural land-use change and its drivers in mountain landscapes: a case study in the Pyrenees. *Agric. Ecosyst. Environ.* 114, 296–310. doi: 10.1016/j.agee.2005.11.017

Nakagawa, S., Johnson, P. C. D., and Schielzeth, H. (2017). The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *J.R. Soc. Interface* 14, 1–11. doi: 10.1098/rsif.2017.0213

Neff, F., Resch, M. C., Marty, A., Rolley, J. D., Schütz, M., Risch, A. C., et al. (2020). Long-term restoration success of insect herbivore communities in seminatural grasslands: a functional approach. *Ecol. Appl.* 30:e02133. doi: 10.1002/eap.2133

Nickel, H. (2003). *The leafhoppers and Planthoppers of Germany (Hemiptera, Auchenorrhyncha): Patterns and strategies in a highly diverse Group of Phytophagous Insects*. Sofia: Pensoft Publishers.

Nickel, H., and Achtziger, R. (1999). Wiesen bewohnende Zikaden (Auchenorrhyncha) im Gradienten von Nutzungsintensität und Feuchte. *Beitr. Zikadenk.* 3, 65–80.

Nickel, H., and Remane, R. (2002). Artenliste der Zikaden Deutschlands, mit Angaben von Nährpflanzen, Nahrungsbreite, Lebenszyklus, Areal und Gefährdung (Hemiptera, Fulgoromorpha et Cicadomorpha). *Beitr. Zikadenk.* 5, 27–64.

Oberdorfer, E. (2001). *Pflanzensoziologische Exkursionsflora für Deutschland und angrenzende Gebiete*, *8th* Edn. Stuttgart: Eugen Ulmer.

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., et al. (2022). Community ecology package (package "vegan," version 2.6–4). Available at: https://cran.r-project.org/web/packages/vegan/vegan.pdf (Accessed 21 October 2022).

Peppler-Lisbach, C., and Petersen, J. (2001). Calluno-Ulicetea (G3), Teil 1: Nardetalia strictae – Borstgrasrasen. *Syn. Pflanzenges. Deutschl.* 8, 1–117.

Pereira, H. M., Navarro, L. M., and Martins, I. S. (2012). Global biodiversity change: the bad, the good, and the unknown. *Annu. Rev. Env. Resour.* 37, 25–50. doi: 10.1146/annurev-environ-042911-093511

Plieninger, T., Höchtl, F., and Spek, T. (2006). Traditional land-use and nature conservation in European rural landscapes. *Environ. Sci. Policy* 9, 317–321. doi: 10.1016/j.envsci.2006.03.001

Poniatowski, D., Beckmann, C., Löffler, F., Münsch, T., Helbing, F., Samways, M. J., et al. (2020). Relative impacts of land-use and climate change on grasshopper range shifts have changed over time. *Glob. Ecol. Biogeogr.* 29, 2190–2202. doi: 10.1111/geb.13188

Poniatowski, D., Löffler, F., Stuhldreher, G., Borchard, F., Krämer, B., and Fartmann, T. (2016). Functional connectivity as an indicator for patch occupancy in grassland specialists. *Ecol. Indic.* 67, 735–742. doi: 10.1016/j.ecolind.2016.03.047

Poniatowski, D., Stuhldreher, G., Löffler, F., and Fartmann, T. (2018). Patch occupancy of grassland specialists: habitat quality matters more than habitat connectivity. *Biol. Conserv.* 225, 237–244. doi: 10.1016/j.biocon.2018.07.018

R Core Team (2022). R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. Available at: https://www.r-project.org/index.html (Accessed 27 April 2022).

Reynolds, D. R., Chapman, J. W., and Stewart, A. J. A. (2017). Windborne migration of Auchenorrhyncha (Hemiptera) over Britain. *Eur. J. Entomol.* 114, 554–564. doi: 10.14411/eje.2017.070

Richards, S. A. (2008). Dealing with overdispersed count data in applied ecology. *J. Appl. Ecol.* 45, 218–227. doi: 10.1111/j.1365-2664.2007.01377.x

Robinson, R., and Sutherland, W. J. (2002). Post-war changes in arable farming and biodiversity in Great Britain. *J. Appl. Ecol.* 39, 157–176. doi: 10.1046/j.1365-2664.2002.00695.x

Roem, W. J., and Berendse, F. (2000). Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities. *Biol. Conserv.* 92, 151–161. doi: 10.1016/S0006-3207(99)00049-X

Rösch, V., Tscharntke, T., Scherber, C., and Batáry, P. (2013). Landscape composition, connectivity and fragment size drive effects of grassland fragmentation on insect communities. *J. Appl. Ecol.* 50, 387–394. doi: 10.1111/1365-2664.12056

Schulte, A. M. (2017). E.05.1 Vegetationskundliches Monitoring zur Restitution/Optimierung von Bergmähwiesen und Borstgrasrasen auf Waldumwandlungs-und Grünlandflächen insbesondere durch Mahdgut-Übertragung. Available at: http://www.bergwiesen-winterberg.de/images/Mon2017_Bericht_mitAnlagen.pdf (Accessed 25 October 2022).

Soliveres, S., van der Plas, F., Manning, P., Prati, D., Gossner, M. M., and Renner, S. C. (2016). Biodiversity at multiple trophic levels is needed for ecosystem multifunctionality. *Nature* 536, 456–459. doi: 10.1038/nature19092

Stevens, C. J., Dise, N. B., Mountford, J. O., and Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science* 303, 1876–1879. doi: 10.1126/science.1094678

Stewart, A. J. A. (2002). Techniques for sampling Auchenorrhyncha in grasslands. *Denisia* 4, 491–512.

Stoate, C., Boatman, N. D., Borralho, R. J., Carvalho, C. R., de Snoo, G. R., and Eden, P. (2001). Ecological impacts of arable intensification in Europe. *J. Environ. Manage.* 63, 337–365. doi: 10.1006/jema.2001.0473

Stöckli, A., Slodowicz, D., Arlettaz, R., and Humbert, J.-Y. (2021). Transfer of invertebrates with hay during restoration operations of extensively managed grasslands in Switzerland. *J. Insect Conserv.* 25, 189–194. doi: 10.1007/s10841-020-00282-8

Strand, L. T., Fjellstad, W., Jackson-Blake, L., and De Wit, H. A. (2021). Afforestation of a pasture in Norway did not result in higher soil carbon, 50 years after planting. *Landscape Urban Plan.* 207:104007. doi: 10.1016/j.landurbplan.2020.104007

Strebel, N., and Bühler, C. (2015). Recent shifts in plant species suggest opposing land-use changes in alpine pastures. *Alp. Bot.* 125, 1–9. doi: 10.1007/s00035-015-0145-3

Stuhldreher, G., and Fartmann, T. (2018). Threatened grassland butterflies as indicators of microclimatic niches along an elevational gradient – implications for conservation in times of climate change. *Ecol. Indic.* 94, 83–98. doi: 10.1016/j.ecolind.2018.06.043

Sullivan, E., Hall, N., and Ashton, P. (2020). Restoration of upland hay meadows over an 11-year chronosequence: an evaluation of the success of green hay transfer. *Restor. Ecol.* 28, 127–137. doi: 10.1111/rec.13063

van Daele, F., Wasof, S., Demey, A., Schelfhout, S., De Schrijver, A., Baeten, L., et al. (2017). Quantifying establishment limitations during the ecological restoration of species-rich *Nardus* grassland. *Appl. Veg. Sci.* 20, 594–607. doi: 10.1111/avsc.12330

van Klink, R., van der Plas, F., van Noordwijk, C. G. E., Wallis DeVries, M. F., and Olff, H. (2015). Effects of large herbivores on grassland arthropod diversity. *Biol. Rev.* 90, 347–366. doi: 10.1111/brv.12113

Wachmann, E., Melber, A., and Deckert, J. (2004). "Wanzen Band 2 – Cimicomorpha: Microphysidae (Flechtenwanzen), Miridae (Weichwanzen)" in *Die Tierwelt Deutschlands*, vol. *75* (Keltern: Goecke & Evers), 1–288.

Wachmann, E., Melber, A., and Deckert, J. (2006). "Wanzen band 1 – Dipsocoromorpha, Nepomorpha, Gerromorpha, Leptopodomorpha, Cimicomorpha (Teil 1)" in *Die Tierwelt Deutschlands*, vol. *77* (Keltern: Goecke & Evers), 1–263.

Wachmann, E., Melber, A., and Deckert, J. (2007). "Wanzen Band 3 – Pentatomomorpha I: Aradidae, Lygaeidae, Piesmatidae, Berytidae, Pyrrhocoridae, Alydidae, Coreidae, Rhopalidae, Stenocephalidae" in *Die Tierwelt Deutschlands*, *Vol. 78* (Keltern: Goecke & Evers), 1–272.

Wachmann, E., Melber, A., and Deckert, J. (2008). "Wanzen band 4 – Pentatomomorpha II, Pentatomoidea: Cydnidae, Thyreocoridae, Plataspidae, Acanthosomatidae, Scutelleridae, Pentatomidae" in *Die Tierwelt Deutschlands*, *Vol. 81* (Keltern: Goecke & Evers), 1–230.

Wagner, M., Hulmes, S., Hulmes, L., Redhead, J. W., Nowakowski, M., and Pywell, R. F. (2020). Green hay transfer for grassland restoration: species capture and establishment. *Restor. Ecol.* 29:e13259. doi: 10.1111/rec.13259

Waldén, E., Öckinger, E., Winsa, M., and Lindborg, R. (2017). Effects of landscape composition, species pool and time on grassland specialists in restored semi-natural grasslands. *Biol. Conserv.* 214, 176–183. doi: 10.1016/j.biocon.2017.07.037

Wilmanns, O. (1998). *Ökologische Pflanzensoziologie*, *6th* Edn. Wiesbaden: Quelle & Meyer.

Zurbrügg, C., and Frank, T. (2006). Factors influencing bug diversity (Insecta: Heteroptera) in semi-natural habitats. *Biodivers. Conserv.* 15, 275–294. doi: 10.1007/s10531-004-8231-7

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 26 May 2023
DOI 10.3389/fevo.2023.1148226

Soil fertility and landscape surrounding former arable fields drive the ecological resilience of Mediterranean dry grassland plant communities

Renaud Jaunatre[1]*, Elise Buisson[2], Elsa Leborgne[1,2] and Thierry Dutoit[2]

[1]Université Grenoble Alpes, INRAE, LESSEM, Saint-Martin-d'Hères, France, [2]IMBE, Avignon University, CNRS, IRD, Aix Marseille University, IUT, Avignon, France

EDITED BY
Orsolya Valkó,
Hungarian Academy of Sciences, Hungary

REVIEWED BY
José Antonio Molina,
Complutense University of Madrid, Spain
Panayiotis G. Dimitrakopoulos,
University of the Aegean, Greece
Florian Delerue,
Institut Polytechnique de Bordeaux, France

*CORRESPONDENCE
Renaud Jaunatre
✉ renaud.jaunatre@inrae.fr

RECEIVED 19 January 2023
ACCEPTED 08 May 2023
PUBLISHED 26 May 2023

CITATION
Jaunatre R, Buisson E, Leborgne E and Dutoit T (2023) Soil fertility and landscape surrounding former arable fields drive the ecological resilience of Mediterranean dry grassland plant communities.
Front. Ecol. Evol. 11:1148226.
doi: 10.3389/fevo.2023.1148226

Introduction: Semi-natural dry grasslands are among the species-richest and most diverse habitats in Europe especially in the Mediterranean Basin. They evolved with long-term severe environmental constraints, either biotic (e.g., grazing or mowing) or abiotic (e.g., dryness or oligotrophy). Their recovery after sever anthropogenic disturbance, such as cultivation is often very slow and incomplete. A deeper understanding of the processes driving the recovery would be of great benefit to better restore or conserve these grasslands. We used the filter model as a framework to assess the relative importance of dispersion, abiotic and biotic filters as drivers of this recovery.

Methods: We studied the medium-term dynamics of 37 Mediterranean dry grassland plant communities after an episode of cultivation in the plain of La Crau (Southern France) by two census: after 25 years of abandonment, and revisited 10 years later (35 years of abandonment). Our aims were to characterize the temporal medium-term dynamics of these communities, to measure their taxonomic resilience and to identify the drivers. We performed structural equation modeling to measure the importance of the various drivers, with cover of remnant dry grasslands surrounding each studied community as a proxy for the dispersion filter, soil pH and phosphorus content as proxies for the abiotic filter and cover of the most dominant species as a proxy for the biotic filter.

Results and Discussion: Our results show that former arable field vegetation do not recover after more than 40 years. The three filters drive the recovery of dry grassland vegetation: former arable fields that have lower phosphorus content (abiotic filter) have a lower cover of dominant species (biotic filter), a higher species richness and a higher similarity with remnant dry grassland plant communities, especially if the former arable fields are surrounded by remnant dry grasslands (dispersion filter).

KEYWORDS
old field, secondary succession, resilience, plant community, dry grassland

1. Introduction

Impacts of land-use changes, notably abandonment of intensive agriculture, are a major topic in conservation biology (Stoate et al., 2009). The conversion from intensive cultivation to extensive grasslands often raises the issue of ecosystem ability to recover their pre-cultivation state of semi-natural species-rich grasslands (Cramer and Hobbs, 2012). This is an important topic as ecosystems recovering after cultivation will cover more than 4 billion ha in the next decades (including both grasslands and forests; Hurtt et al., 2011). Secondary succession is an essential concern in plant ecology (Cramer et al., 2008; Cramer and Hobbs, 2012), not only for theoretically-driven studies (Connell and Slatyer, 1977; Prach and Walker, 2011; Seabloom et al., 2020), but also for conservation and restoration perspectives (Luken, 1990; Walker et al., 2007; Suding, 2011; Rapson, 2023).

Species-rich grasslands are rarely resilient to severe anthropogenic disturbances (Helm et al., 2019; Nerlekar and Veldman, 2020; Buisson et al., 2022). They are composed with species that developed strategies to survive in moderately stressed and/or disturbed environment (i.e., xericity, grazing, mowing, combined or not) but not to recover after severe disturbance (Herben et al., 2018), such as intensive arable cultivation which involve soil disturbance as well as nutrient, herbicide and pesticide additions. In order to restore these degraded ecosystems it would be useful to better understand the drivers of recovery (i.e., their relative importance, their magnitude and directions). These drivers could then be used as levers that practitioners can modify.

Processes have been linked to theoretical models in order to summarize how communities assemble (Keddy, 1992; Lortie et al., 2004; Vellend, 2016). Most of these models are structured by filters that constrain community composition starting from a regional species pool. The first filter is dispersion: species have to be able to disperse and reach the community. This ability depends both on the landscape matrix and on the dispersability of species found in adjacent areas (Gibson and Brown, 1991; Pärtel and Zobel, 1999; Lindborg and Eriksson, 2004; Herault and Thoen, 2009). The second is the abiotic filter: species have to be able to germinate, grow and reproduce under given environmental conditions, and this ability depends on each species physiological capability (Kraft et al., 2015). The last one is the biotic filter: the occurrence and nature of biotic interactions alter the first two filters either by enhancing their mesh (*sensu* White and Jentsch, 2004), e.g., by increasing dispersion or by facilitating growth or establishment in limiting environmental conditions; or by reducing their mesh, e.g., by competitive exclusion (Bruno et al., 2003; Lortie et al., 2004). The aim of this paper is to disentangle the effect of dispersion, abiotic and biotic filters in driving the recovery of species-rich dry grassland plant communities after decades of intensive cultivation abandonment.

In this study, we measured diversity and composition recoveries in 35 and 45 year-old former arable fields in Mediterranean semi-natural species-rich dry grasslands and we have a hypothesis for each filter. The amount of remnant ecosystem in the surrounding of the former arable fields was used as a proxy of the dispersion filter as it provides a seed source for species occurring in the dry grassland. Our hypothesis was that its importance was rather low in driving plant communities. It is indeed widely recognized that most of the species from dry grasslands do not produce a permanent seed bank or seed that disperse on large distances (Graham and Hutchings, 1988; Buisson et al., 2006; Török et al., 2018; Arruda et al., 2021). Considering the size of the former arable fields (>100,000 m^2) and the fact that most of the species do not colonize more than a few meters after decades (Buisson and Dutoit, 2004, 2006), these species are not expected to disperse to the whole area of each former arable fields, whatever the amount of remnant ecosystem in the surroundings. Two soil variables that were modified during former cultivation episode were used as proxies for the abiotic filter: one is related to fertility (phosphorus content) and the other is not directly related to soil fertility (soil pH). Phosphorus is one of the most important abiotic drivers of grassland community assembly (Yang et al., 2022). It is also known to remain in soil decades after fertilized cultivation, contrary to potassium or nitrogen (Smits et al., 2008; Jaunatre et al., 2016; Saatkamp et al., 2021). Ploughing which scraped the underlying calcareous conglomerate (Römermann et al., 2005), changed soil chemistry, increasing CaO, CaCO3 and pH. We thus selected pH as a proxy of these changes, as they may prevent the plant community to recover the composition of remnant dry grasslands [e.g., pH contributes to explain plant species distribution (Dubuis et al., 2013)]. Our hypotheses were that soil pH would influence plant community composition as soil pH is also a soil physical-parameter integrative of others changes than chemical fertility (i.e., increase of Ca and CaCO3 contents) while phosphorus content would decrease diversity through an increase in competition (Janssens et al., 1998; Palpurina et al., 2019). The biotic filter may depend on the abiotic characteristics (Belyea, 2004). During former arable field recovery, plant species have to face dense cover of ruderals or opportunistic species (Baeten et al., 2009; Öster et al., 2009). Hence, we used the dominant species cover as an indicator of potential competition in former arable fields and thus as a proxy of the biotic filter. Our hypothesis was that phosphorus content would drive the dominant species cover which itself would drive the plant community diversity. We also hypothesized that high composition recovery would only occur when species richness has recovered. The composition of the community could indeed be different from that of the remnant dry grasslands if the number of species is lower.

We monitored vegetation on 37 former arable field plots and 10 remnant dry grassland plots (in 2009 and in 2019), respectively 35 and 45 years after cultivation abandonment. We also characterized soil physico-chemical parameters on each plot and the landscape in the surroundings of each plot. In order to link the different drivers of plant community assembly we performed a structural equation modeling for each date were filter proxies were explanatory variables and vegetation diversity and composition were response variables.

2. Materials and methods

2.1. Site description

The La Crau area is a large dry grassland in southeastern France (ca. 10,000 ha; c. 43°33′ N, 4°52′ E; Figure 1) that has been shaped by (i) a Mediterranean climate with mean annual temperature of 15°C, a variable annual sum of precipitation between 400 and 600 mm concentrated in autumn, 4 months of summer drought, and more than 110 days with a >50 km.h-1 wind; (ii) 40 cm deep soil composed with about 50% of silicaceous stones overlaying a calcareous conglomerate layer, making the alluvial water table unreachable by the roots of plants and (iii) itinerant sheep that have grazed the area over the past several 1000 years (Devaux et al., 1983; Buisson and Dutoit, 2006). This has led to a unique and species-rich plant community composed mainly by a high number of annuals yet dominated in biomass by perennials, such as *Brachypodium retusum* Pers. and *Thymus vulgaris* L. Most of the species occurring in La Crau area are species adapted to extensive grazing, xeric and oligotrophic conditions. This community characterizes the remnant ecosystem in this study (i.e., that has been grazed for millennia but that has not undergone any disturbance due to cultivation) and will henceforth be referred to as dry grasslands. The

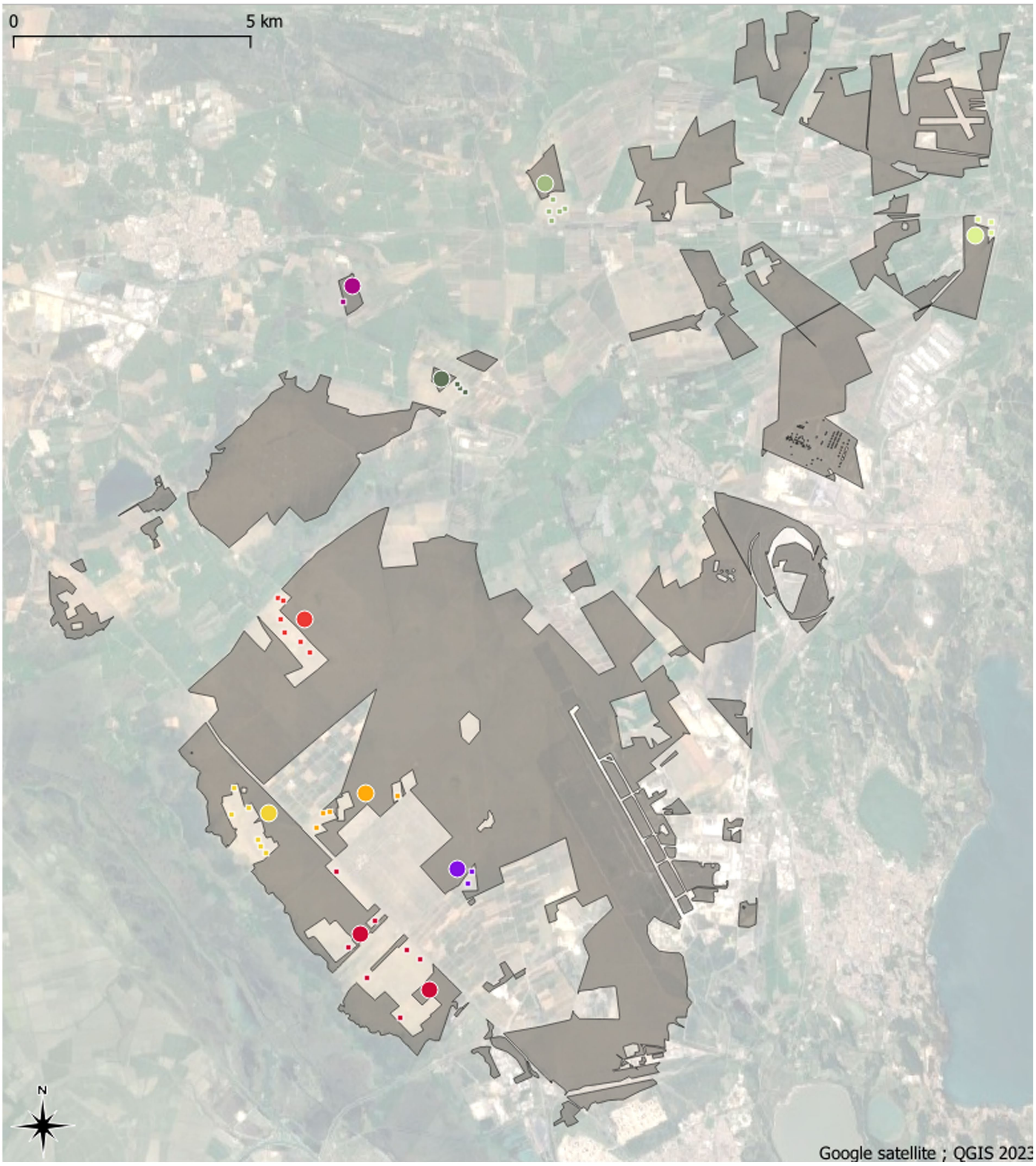


FIGURE 1
Map of the La Crau area in 2023. Remnant dry grasslands habitats are shown in grey. Colored large circles are remnant dry grasslands plots whereas colored small squares are former arable field plots. The different geographical areas are discriminated by different colors that are used in the ordination plot (Figure 4).

60,000 ha of original dry grasslands was characterized by relatively homogeneous vegetation composed only of different micro-patterns due to particular soil conditions and difference in sheep grazing regimes (Martin et al., 2022), before being fragmented by various severe anthropogenic disturbances, in particular, cultivation (Devaux et al., 1983; Buisson and Dutoit, 2006). Melon cultivation occurred in the La Crau area between 1971 and 1988. This cultivation implied soil disturbance, plant mortality and fertilizers application. Most of the fields have been subsequently abandoned; they now contain various plant communities dominated by *Anisantha* species (Römermann et al., 2005).

2.2. Site selection

Mapping based on aerial photographs enabled us to identify more than 220 areas that (i) appear as dry grasslands on the earliest photographs available (1947); (ii) show evidence of cultivation in later photographs (1955–1971–1975–1978–1979–1984–1988); and (iii) were abandoned between 1978 and 1988. After abandonment, all these areas were grazed again with the same intensity as the remnant dry grasslands. To avoid the introduction of an additional source of variability, we focused only on former open-field melon cultivation and not on large plastic tunnel fields that left less-disturbed trackways between the tunnels (Römermann et al., 2005). Thirty-seven of these Former Arable Fields, hereafter called FAF, were chosen and are spatially randomly distributed. The distance between any two sampled sites varies between 100 m and 20.9 km (Figure 1).

2.3. Vegetation sampling

Vegetation sampling was carried out at the center of each of the 37 FAF and of 10 dry grassland areas from all geographical zones of the La Crau area (Figure 1) in 2009 and in 2019, by the same team of botanists. On each FAF and remnant dry grassland areas, we monitored the vegetation in three 4 m^2 quadrats at the tips of an 11 m side triangle. A Braun-Blanquet coefficient (Braun-Blanquet et al., 1952) was given to all plant species recorded and then transformed into percentage according to Guinochet (1973; 5: 87.5%, 4: 62.5%, 3: 37.5%, 2: 17.5%, 1: 5% and +: 0.1%). To avoid biases in abundance attributions, we transformed these percentages into relative percentage to the total vegetation cover. By doing so, we keep abundance hierarchy but we lever the potential overlap between species cover by constraining the sum of abundances into the total vegetation cover. We used species richness (alpha diversity) and similarity to the remnant dry grassland communities (composition) as response variables. The similarity to the remnant dry grassland communities was calculated as 1 – the minimum Bray-Curtis distance (Bray and Curtis, 1957) with the remnant dry grassland plot communities. We used the minimum Bray-Curtis distance instead of the average value in order to reduce the effect of the heterogeneity of remnant dry grassland vegetation. This similarity index with remnant dry grassland communities was thereafter referred as composition.

2.4. Explanatory variables

2.4.1. Remnant dry grassland cover

We used remnant dry grassland percent cover within a 900 m radius circle around the centre of each field as a proxy of dispersion potential. Map of remnant dry grassland areas was provided by the national reserve environmental managers (Réserve Nationale des Coussouls de Crau, Wolff et al., 2015). The 900 m radius was chosen based on preliminary analyses showing a tradeoff between (i) a smaller radius that would have caused the landscape factors to be especially influenced by the area of each field, and (ii) a larger radius that would have made the landscape factors of two fields close together almost identical. Cover were calculated using Geographical Information System software: Quantum GIS 2.18 “Las Palmas” (QGIS Development Team, 2016).

2.4.2. Soil analyses

We used soil analyses as a proxy of abiotic filter. For soil analyses, in 2009 and in 2019, a subsample of 70 g of soil was gathered in each of the three vegetation quadrats in the topsoil layer (0-10 cm) before being pooled to obtain one 210 g soil sample for each FAF. Samples were then sieved with a 2 mm mesh sieve. Analyses were carried out by the INRAE (Institut National de Recherche pour l’Agriculture l’Alimentation et l’Environnement). Phosphorus content (P2O5; Olsen et al., 1954) and water pH were measured according to the standard methods described in Baize (2018).

2.4.3. Dominance

We used the dominance as a proxy of the biotic filter. Dominance was calculated as the sum of abundances of the four most abundant species in each quadrat, which represented in average 45% of the sum of all species abundances (Appendix 1).

2.5. Statistical analyses

All the analyses were conducted using R 4.2.2 (R Core Team, 2022).

The alpha diversity of remnant dry grasslands and FAF were compared using a generalized linear mixed model performed with LME4 package (Bates et al., 2015). The kind of plant community (remnant dry grasslands or FAF) and monitoring years were included as fixed factors and the monitoring years and plot interaction as random factor to take into account the repeated nature of data.

In order to describe the vegetation of dry grasslands and FAF together, a Non-metric Multidimensional Scaling (NMDS) ordination was performed on the vegetation matrix to ordinate quadrats according to their plant community characteristics (Borcard et al., 2011) with VEGAN package (Oksanen et al., 2022). We performed a permutational analysis of variance using Bray-Curtis distance matrices (McArdle and Anderson, 2001), with the VEGAN::ADONIS2 function. This analysis measures the difference in the position of the centroid by groups, but may confound location and dispersion effects (Warton et al., 2012), we therefore also performed a test of homogeneity of group dispersion (Anderson, 2006) with the VEGAN::BETADISPER function.

In order to understand how the variation of landscape context, soil parameters and cover of dominant species influence the diversity and composition (i.e., similarity to remnant dry grassland areas) of FAF, we built a structural equation model (SEM) with PIECEWISESEM package (Lefcheck, 2016) for each monitoring year (2009 and 2019). This analysis, based on pathway dependence between variables, makes it possible to identify hypothesized relationships between selected variables (Lefcheck, 2016). We included dominance, alpha diversity and composition as response variables and remnant dry grassland cover, phosphorus content, pH and dominance as predictor variables for each plot (Figure 2). We fitted the SEMs with mixed models using the predictor variables as fixed factors and plots as random factor. For dominance, we used a linear model, with LMERTEST package (Kuznetsova et al., 2017), after square root transformation in order to fulfill parametric conditions. For alpha diversity we used a generalized linear model, with LME4 package (Bates et al., 2015), with poisson family and log link function. For composition, we used linear model, with LMERTEST package (Kuznetsova et al., 2017). Once fitted, we added missing pathways if they had been highlighted by Fisher d-separation tests (Shipley, 2013). We chose the best SEM model as the one with no significant missing pathways and with the lowest

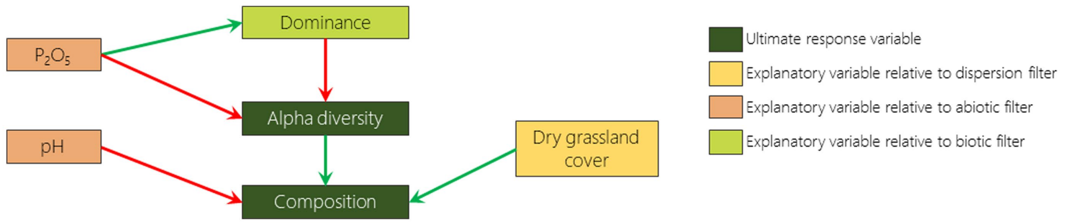


FIGURE 2
Pathway diagram representing the hypotheses tested in the structural equation modelling. Each arrow represents an expected effect, positive in green and negative in red.

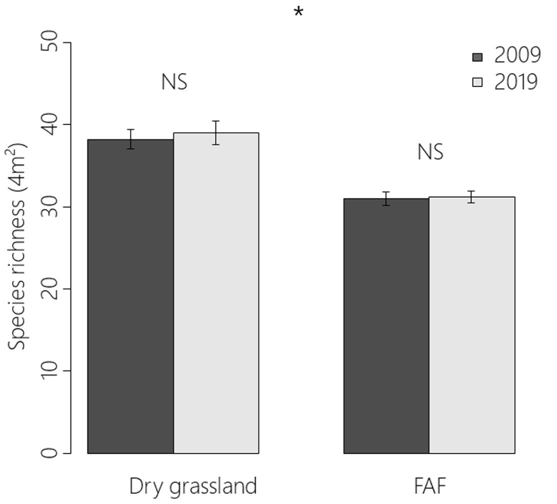


FIGURE 3
Means and standard errors of species-richness (4 m²) for 2009 and 2019 in remnant dry grasslands and former arable field plots (GLMM; $z = -2.49$, $p = 0.013$). "NS" indicated that the effect was not significant and "*" that it was with a p value between 0.05 and 0.01 according to a generalized linear mixed model.

Akaike information criterion by at least 2 units (AIC; Akaike, 1974; Burnham and Anderson, 2004; Appendix 1).

3. Results

In total, 172 plant species were recorded: 156 in 222 FAF quadrats (37 × 3 × 2 quadrats) and 127 in 60 remnant dry grassland quadrats (10 × 3 × 2 quadrats). Despite the high number of species in common between remnant dry grasslands and FAF plots, mean species richness was significantly higher in remnant dry grassland areas than in FAFs (38.6 ± 0.9 vs. 31.1 ± 0.5; $z = -2.49$, $p = 0.013$; Figure 3). There was however neither an effect of monitoring year ($z = 0.292$; $p = 0.771$) nor an effect of interaction between year and location ($z = -0.011$; 0.992).

There were no clear tendencies for the composition trajectories between 2009 and 2019 (Figure 4), although there is a significant year effect on the position of the centroid (F1 = 15.06; $p < 0.001$) with no difference of dispersion (F1 = 0.16; $p = 0.686$). On the contrary, remnant dry grasslands and FAF have a significantly different position of centroids (F1 = 24.25; $p < 0.001$) associated with a significant difference of dispersion (F1 = 88.98; $p < 0.001$). Some FAFs are indeed embedded within remnant dry grassland vegetation and characterized by species, such as *Brachypodium retusum* P. Beauv., *Thymus vulgaris* L., *Medicago* spp. and *Trifolium* spp. while others are still discriminated from the remnant dry grasslands and are characterized by the dominance of more ruderal type species for the Crau area, such as *Lobularia maritima* (L.) Desv., *Anisantha madritensis* (L.) Nevski or *Clinopodium nepeta* (L.) Kuntze.

According to the best fitted SEM, 2 out of 6 hypothesized relationships were confirmed for 2009 and 2019: the negative relationship between dominance and alpha diversity and the positive relationship between alpha diversity and composition (Figures 5, 6). The hypothesized negative relationship between pH and composition was confirmed in none of the models. Other relationships depended on the monitoring year. Phosphorus content was positively correlated (although non significantly) with dominance in 2009 and negatively correlated with alpha diversity in 2019. The remnant dry grassland cover had a positive relationship with composition in 2019 only. Surprisingly, a negative relationship that was not included in our *a priori* hypotheses was identified as missing pathways by Fisher d-separation tests (Shipley, 2013) between remnant dry grassland cover and dominance in 2009 (Figure 5).

4. Discussion

As reported in many studies that have focused on plant community resilience (Tomanek et al., 1955; Bonet and Pausas, 2004; Jaunatre et al., 2016; Helm et al., 2019; Isbell et al., 2019), even more than 30 years after disturbance, the species richness of the plant communities that colonized the disturbed sites are still lower than, and the composition is still considerably different from, those from the remnant dry grasslands. When we disentangle the different drivers of vegetation, we first notice that in 2009 phosphorus content and remnant dry grassland cover in the surroundings increase the abundance of the most dominant species in the FAF (e.g., *Anisantha* spp.). This dominance appears to reduce alpha diversity which itself drives the composition (i.e., a lower species richness leads to a lower similarity to the remnant dry grasslands). We then note that in 2019 while alpha diversity is reduced by dominance, it is also reduced by phosphorus content. Finally, we measured that while composition is driven by alpha diversity, it is also driven by the cover of dry grasslands in the surroundings (i.e., a higher cover of remnant dry grasslands in the surrounding leads to a higher similarity with these dry grasslands). It is therefore interesting to stress that the three filters play an

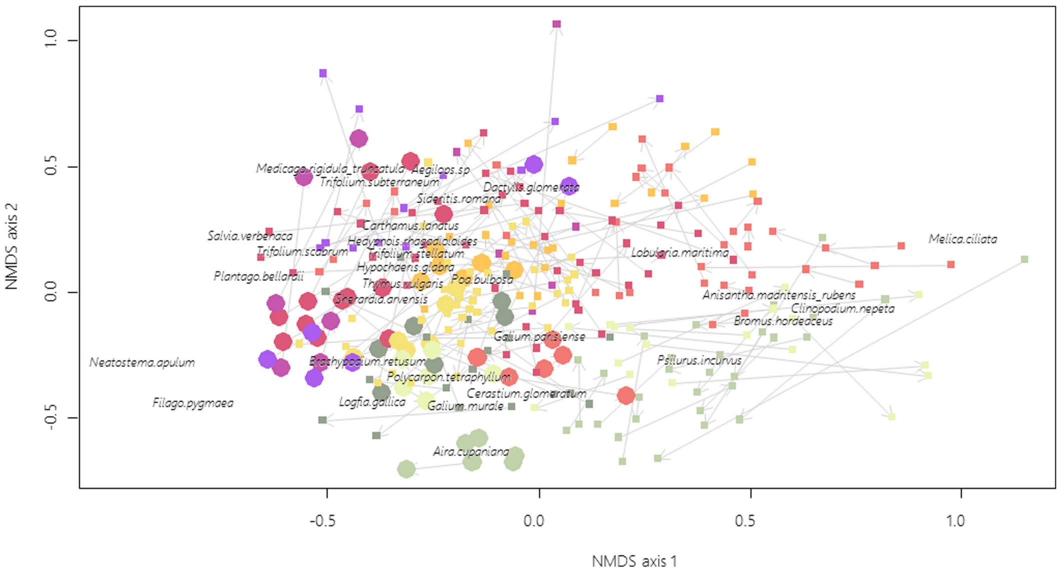


FIGURE 4
Remnant dry grasslands (filled circles, 10 sites × 3 plots × 2 years) and FAF (filled squares, *n* = 37 FAF × 3 plots × 2 years) ordination based on NMDS of plant community composition, final stress=0.28. For clarity purpose, only the 30 most correlated species are shown (out of 172). Arrows link 2009 plots to 2019 plots. Points with the same colors are located within the same geographical zones (Figure 1).

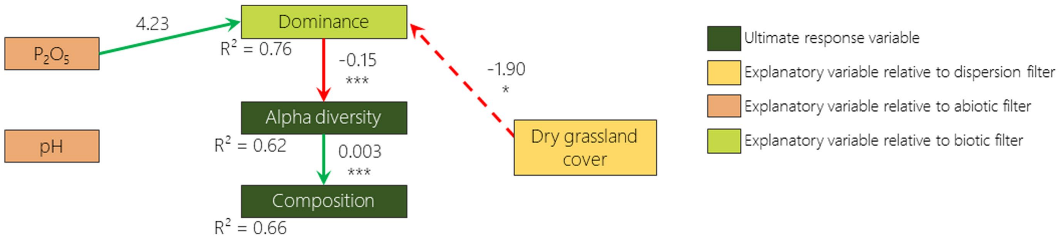


FIGURE 5
2009 survey pathway diagram of the best fitted SEM illustrating how dominance, alpha diversity and composition are influenced by remnant dry grasslands cover, phosphorus content, pH and dominance. Boxes represent responses and predictor variables. Only included links in this model are drawn with arrows (green if the relationship is positive and red if negative). Dotted arrows represent links that were not included in our *a priori* hypotheses but were added after looking for missing pathways by Fisher d-separation tests (Shipley, 2013). Numbers on arrows are the slope estimates, accompanied or not with stars (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

important role in driving the vegetation recovery, but not with the same processes.

Surprisingly, the dispersion proxy (remnant dry grassland cover) switched from being negatively correlated with dominance in 2009 to being positively correlated with composition in 2019. A possible explanation of this result, is that when remnant dry grassland cover is low, cultivated area or former arable field covers are high. Differential dispersal limitation can cause wide species-specific deviations in the probability of finding a species from the landscape species pool in the community (Grace, 2001). Fields surrounded by cultivated areas or former arable fields may therefore exhibit a higher abundance of anemochorous ruderal species like *Anisantha* spp. that are known to exhibit higher dispersion abilities (Gaba et al., 2017). Ten years later, the presence of remnant dry grasslands in the vicinity promotes a composition more similar to the dry grassland. It is well known that presence of remnant patches in the vicinity promotes the dispersal of remnant dry grassland species to the community (Tansley and Adamson, 1925) and that this effect increases with time (Cook et al., 2005). We had not hypothesized a high importance of remnant patches as most of the species from dry grasslands do not disperse on large distances due to the low quantity of seeds from remnant communities in the seed bank and the seed rain (Graham and Hutchings, 1988; Buisson and Dutoit, 2004; Török et al., 2018; Arruda et al., 2021). Nevertheless, on the long term (over 30 years), long distance dispersion events (i.e., hundreds of meters) with low probability (i.e., which was not detected by sampling design) can occur (Higgins and Richardson, 1999) and may be favored by the presence of remnant patches in the vicinity. This is supported by the fact that FAF tend to differ from each other in patterns similar to those of the dry grasslands. Such patterns are also observed on secondary

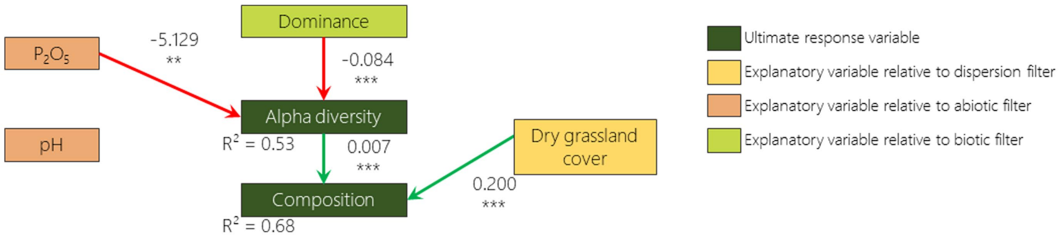


FIGURE 6
2019 survey pathway diagram of the best fitted SEM illustrating how dominance, alpha diversity and composition are influenced by remnant dry grasslands cover, phosphorus content, pH, and dominance. Boxes represent responses and predictor variables. Only included links in this model are drawn with arrows (green if the relationship is positive and red if negative). Numbers on arrows are the slope estimates, accompanied or not with stars (*$p<0.05$; **$p<0.01$; ***$p<0.001$).

succession in abandoned mining sites in Central Europe (Jírová et al., 2012). However, the design of our study does not allow us to differentiate whether this pattern is a result of the dispersion or the abiotic filters. A more in depth spatial characterization of the remnant dry grasslands would be required to disentangle the effect of the species-pool it provides and the abiotic spatial organization. In order to thoroughly tackle the dispersion filter, numerous census of occurring species would be required as well as the correspondence with dispersal traits of these species (Lewis et al., 2016).

The pH does not appear to exert an effect on composition, which was not expected by our hypotheses. We expected higher changes due to the calcareous conglomerate that was broken during the cultivation phase (Buisson and Dutoit, 2004) and therefore lower composition recovery of plant community compared to remnant dry grasslands that contains some acidophilous species (Devaux et al., 1983). The range of variation of pH was however rather low: 6.9 and 7.2 being, respectively, the first and third quartiles. If relationship between pH and composition should occur, a higher range of variation would be require to detect it. Phosphorus content has first a positive relationship with dominance and then with species richness. Higher fertility leads to higher biomass of annual grasses (higher dominance) that should have increased competition (Michalet et al., 2023). Nutrient enrichment does not prevent remnant dry grassland species development in itself but enhances competition of more generalist species due to their dense cover (Huenneke et al., 1990; Buisson et al., 2015). Sowing typical remnant dry grassland species in FAF abiotic conditions or in remnant dry grassland abiotic conditions does not influence their establishment (Coiffait-Gombault et al., 2012). Our results show that species richness is limited both by biotic (dominance) and abiotic (phosphorus content) filters that interact together. This is in accordance with previous studies that show that dominance by more competitive and ruderal species on soil with higher nutrient contents can lead to a decline of remnant community species (Yurkonis and Meiners, 2004; Seabloom et al., 2020). Apart from the important feedback with the abiotic filter, the biotic filter is complicated to take into account. In our study, we only focused on the dominance of the above ground vegetation. The competition between plants may also be tackled by measurement of functional trait patterns, but inferring competition processes from these patterns may sometimes be misleading (Münkemüller et al., 2020). Besides intra guild competition, there is also below ground interactions (Kiær et al., 2013), facilitative interactions (Brooker et al., 2007), soil microfauna (Sun et al., 2023), microbial communities (Kaisermann et al., 2017), etc. We are currently able to measure the importance of these trophic network in the driving of ecosystem functioning (Martinez-Almoyna et al., 2022), but it is still very difficult to disentangle all the direct and indirect interactions that could lead to the favoring or (disfavoring) of specific set of species.

We studied the recovery on the vegetation composition having as reference, remnant patches of remnant dry grassland vegetation (i.e., extensively grazed vegetation). Considering the vegetation dynamics that we measured after several decades of recovery, it is unlikely that these plant communities will converge toward remnant dry grassland vegetation on the medium term. As was shown in previous studies, dozens of decades after abandonment, the composition is still different from the remnant ecosystems (Jaunatre et al., 2016, for species-rich dry grassland; Nerlekar and Veldman, 2020 for various grasslands). For such non-resilient species-rich grasslands, reconstructive restoration (i.e., ending degradation, active remediation of substrate condition and reintroduction of major proportion of target biota *sensu* Atkinson and Bonser, 2020) would be needed to ensure a potential complete recovery of plant communities. Our result confirm the fact that the restoration of abiotic conditions prior to forcing the dispersion of target species would be a key for successful restoration.

Our results show that FAF vegetation did not recover after more than 30 years. The three filters drive the recovery of remnant dry grassland vegetation: FAF that have lower phosphorus content (abiotic filter) have a lower cover of dominant species (biotic filter), a higher species richness and a higher similarity with remnant dry grassland plant communities, especially if the FAF are surrounded by remnant dry grasslands (dispersion filter). Based on these results we therefore recommend that the restoration of species-rich semi-natural remnant dry grasslands is primarily focused on the recovery of suitable abiotic conditions [i.e., by decreasing fertility, for instance with expensive topsoil removal (Hölzel and Otte, 2003; Jaunatre et al., 2014), repeated carbon addition (Corbin and D'Antonio, 2004; Ossanna and Gornish, 2023) or long-term phosphorus mining (Schelfhout et al., 2015)] and on increasing propagule pressure of the typical remnant dry grassland species in a second phase. In order to be able to use SEM, we only used a few variables that we attributed to the three filters. These variables were chosen regarding the specific characteristics of the degradation and of the remnant ecosystem. Our study is a piece into the comprehension of the relative importance of each filters that would benefit from both similar analyses in other ecosystems but also more in depth measure of how the propagule pressure and the abiotic characteristics drive interaction between species and therefore plant community recovery.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author. Code and data are also publicly available at https://forgemia.inra.fr/renaud.jaunatre/friches_frontiers.

Author contributions

RJ, EB, and TD conceived the design of this study. RJ performed the data analysis and wrote the first draft of the manuscript. All authors contributed to floristic surveys and approved the final version.

Funding

This study was supported by the CDC Biodiversité, CEN PACA, the Réserve Naturelle des Coussouls de Crau, the Conseil Régional de Provence Alpes Côtes d'Azur, and CNRS RTP Ingecotech funding.

Acknowledgments

The authors would like to thank Jennifer Matwyschuk and Daniel Pavon (IMBE) for help with fieldwork.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1148226/full#supplementary-material

References

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* 19, 716–723. doi: 10.1109/TAC.1974.1100705

Anderson, M. J. (2006). Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62, 245–253. doi: 10.1111/j.1541-0420.2005.00440.x

Arruda, A. J., Junqueira, P. A., Rodrigues, H. T. S., Yvanez, F., Poschlod, P., Silveira, F. A. O., et al. (2021). Limited seed dispersability in a megadiverse OCBIL grassland. *Biol. J. Linn. Soc.* 133, 499–511. doi: 10.1093/biolinnean/blaa145

Atkinson, J., and Bonser, S. P. (2020). "Active" and "passive" ecological restoration strategies in meta-analysis. *Restor. Ecol.* 28, 1032–1035. doi: 10.1111/rec.13229

Baeten, L., Jacquemyn, H., Van Calster, H., Van Beek, E., Devlaeminck, R., Verheyen, K., et al. (2009). Low recruitment across life stages partly accounts for the slow colonization of forest herbs. *J. Ecol.* 97, 109–117. doi: 10.1111/j.1365-2745.2008.01455.x

Baize, D. (2018). *Guide des analyses en pédologie. 3e éd. revue et augmentée* Versailles: Éditions Quae.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* 67, 1–48. doi: 10.18637/jss.v067.i01

Belyea, L. R. (2004). "Beyond ecological filters: feedback networks in the assembly and restoration of community structure" in *Assembly rules and restoration ecology: bridging the gap between theory and practice*. eds. V. M. Temperton, R. J. Hobbs, T. Nuttle and S. Halle, (Washington, DC: Island Press), 115–131.

Bonet, A., and Pausas, J. G. (2004). Species richness and cover along a 60-year chronosequence in old-fields of southeastern Spain. *Plant Ecol.* 174, 257–270. doi: 10.1023/B:VEGE.0000049106.96330.9c

Borcard, D., Gillet, F., and Legendre, P. (2011). *Numerical ecology with R* New York, NY: Springer.

Braun-Blanquet, J., Roussine, N., and Nègre, R. (1952). *Groupements végétaux de la France méditerranéenne*. CNRS. Montpellier.

Bray, J. R., and Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* 27, 325–349. doi: 10.2307/1942268

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., et al. (2007). Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* 96, 18–34. doi: 10.1111/j.1365-2745.2007.01295.x

Bruno, J. F., Stachowicz, J. J., and Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* 18, 119–125. doi: 10.1016/S0169-5347(02)00045-9

Buisson, E., Archibald, S., Fidelis, A., and Suding, K. N. (2022). Ancient grasslands guide ambitious goals in grassland restoration. *Science* 377, 594–598. doi: 10.1126/science.abo4605

Buisson, E., Corcket, E., and Dutoit, T. (2015). Limiting processes for perennial plant reintroduction to restore dry grasslands: perennial plant reintroduction in dry grasslands. *Restor. Ecol.* 23, 947–954. doi: 10.1111/rec.12255

Buisson, E., and Dutoit, T. (2004). Colonisation by native species of abandoned farmland adjacent to a remnant patch of Mediterranean steppe. *Plant Ecol.* 174, 371–384. doi: 10.1007/BF03543067

Buisson, E., and Dutoit, T. (2006). Creation of the natural reserve of La Crau: implications for the creation and management of protected areas. *J. Environ. Manag.* 80, 318–326. doi: 10.1016/j.jenvman.2005.09.013

Buisson, E., Holl, K. D., Anderson, S., Corcket, E., Hayes, G. F., Torre, F., et al. (2006). Effect of seed source, topsoil removal, and plant neighbor removal on restoring California coastal prairies. *Restor. Ecol.* 14, 569–577. doi: 10.1111/j.1526-100X.2006.00168.x

Burnham, K. P., and Anderson, D. R. eds. (2004). *Model selection and multimodel inference*. New York, NY: Springer.

Coiffait-Gombault, C., Buisson, E., and Dutoit, T. (2012). Using a two-phase sowing approach in restoration: sowing foundation species to restore, and subordinate species to evaluate restoration success. *Appl. Veg. Sci.* 15, 277–289. doi: 10.1111/j.1654-109X.2012.01182.x

Connell, J. H., and Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *Am. Nat.* 111, 1119–1144. doi: 10.1086/283241

Cook, W. M., Yao, J., Foster, B. L., Holt, R. D., and Patrick, L. B. (2005). Secondary succession in an experimentally fragmented landscape: community patterns across space and time. *Ecology* 86, 1267–1279. doi: 10.1890/04-0320

Corbin, J. D., and D'Antonio, C. M. (2004). Can carbon addition increase competitiveness of native grasses? A case study from California. *Restor. Ecol.* 12, 36–43. doi: 10.1111/j.1061-2971.2004.00299.x

Cramer, V. A., and Hobbs, R. (2012). *Old fields: dynamics and restoration of abandoned farmland*. Washington, DC: Island Press.

Cramer, V., Hobbs, R., and Standish, R. (2008). What's new about old fields? Land abandonment and ecosystem assembly. *Trends Ecol. Evol.* 23, 104–112. doi: 10.1016/j.tree.2007.10.005

Devaux, J. P., Archiloque, A., Borel, L., Bourrelly, M., and Louis-Palluel, J. (1983). Notice de la carte phyto-écologique de la Crau (Bouches du Rhône). *Biologie et écologie méditerranéenne* 10, 5–54.

Dubuis, A., Rossier, L., Pottier, J., Pellissier, L., Vittoz, P., and Guisan, A. (2013). Predicting current and future spatial community patterns of plant functional traits. *Ecography* 36, 1158–1168. doi: 10.1111/j.1600-0587.2013.00237.x

Gaba, S., Perronne, R., Fried, G., Gardarin, A., Bretagnolle, F., Biju-Duval, L., et al. (2017). Response and effect traits of arable weeds in agro-ecosystems: a review of current knowledge. *Weed Res.* 57, 123–147. doi: 10.1111/wre.12245

Gibson, C. W. D., and Brown, V. K. (1991). The nature and rate of development of calcareous grassland in southern Britain. *Biol. Conserv.* 58, 297–316. doi: 10.1016/0006-3207(91)90097-S

Grace, J. B. (2001). Difficulties with estimating and interpreting species pools and the implications for understanding patterns of diversity. *Folia Geobot.* 36, 71–83. doi: 10.1007/BF02803140

Graham, D. J., and Hutchings, M. J. (1988). Estimation of the seed bank of a chalk grassland ley established on former arable land. *J. Appl. Ecol.* 25, 241–252. doi: 10.2307/2403622

Guinochet, M. (1973). *Phytosociologie*. Paris: Masson.

Helm, J., Dutoit, T., Saatkamp, A., Bucher, S. F., Leiterer, M., and Römermann, C. (2019). Recovery of Mediterranean steppe vegetation after cultivation: legacy effects on plant composition, soil properties and functional traits. *Appl. Veg. Sci.* 22, 71–84. doi: 10.1111/avsc.12415

Herault, B., and Thoen, D. (2009). How habitat area, local and regional factors shape plant assemblages in isolated closed depressions. *Acta Oecol.* 35, 385–392. doi: 10.1016/j.actao.2009.02.002

Herben, T., Klimešová, J., and Chytrý, M. (2018). Philip Grime's fourth corner: are there plant species adapted to high disturbance and low productivity? *Oikos* 127, 1125–1131. doi: 10.1111/oik.05090

Higgins, S. I., and Richardson, D. M. (1999). Predicting plant migration rates in a changing world: the role of long-distance dispersal. *Am. Nat.* 153, 464–475. doi: 10.1086/303193

Hölzel, N., and Otte, A. (2003). Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. *Appl. Veg. Sci.* 6, 131–140. doi: 10.1111/j.1654-109X.2003.tb00573.x

Huenneke, L. F., Hamburg, S. P., Koide, R., Mooney, H. A., and Vitousek, P. M. (1990). Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology* 71, 478–491. doi: 10.2307/1940302

Hurtt, G. C., Chini, L. P., Frolking, S., Betts, R. A., Feddema, J., Fischer, G., et al. (2011). Harmonization of land-use scenarios for the period 1500–2100: 600 years of global gridded annual land-use transitions, wood harvest, and resulting secondary lands. *Clim. Chang.* 109, 117–161. doi: 10.1007/s10584-011-0153-2

Isbell, F., Tilman, D., Reich, P. B., and Clark, A. T. (2019). Deficits of biodiversity and productivity linger a century after agricultural abandonment. *Nat. Ecol. Evol.* 3, 1533–1538. doi: 10.1038/s41559-019-1012-1

Janssens, F., Peeters, A., Tallowin, J. R. B., Bakker, J. P., Bekker, R. M., Fillat, F., et al. (1998). Relationship between soil chemical factors and grassland diversity. *Plant Soil* 202, 69–78. doi: 10.1023/A:1004389614865

Jaunatre, R., Buisson, E., and Dutoit, T. (2014). Topsoil removal improves various restoration treatments of a Mediterranean steppe (La Crau, Southeast France). *Appl. Veg. Sci.* 17, 236–245. doi: 10.1111/avsc.12063

Jaunatre, R., Fonvieille, N., Spiegelberger, T., Buisson, E., and Dutoit, T. (2016). Recovery of arbuscular mycorrhizal fungi root colonization after severe anthropogenic disturbance: four species assessed in old-growth Mediterranean grassland. *Folia Geobot.* 51, 319–332. doi: 10.1007/s12224-016-9254-z

Jírová, A., Klaudisová, A., and Prach, K. (2012). Spontaneous restoration of target vegetation in old-fields in a central European landscape: a repeated analysis after three decades. *Appl. Veg. Sci.* 15, 245–252. doi: 10.1111/j.1654-109X.2011.01165.x

Kaisermann, A., de Vries, F. T., Griffiths, R. I., and Bardgett, R. D. (2017). Legacy effects of drought on plant–soil feedbacks and plant–plant interactions. *New Phytol.* 215, 1413–1424. doi: 10.1111/nph.14661

Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *J. Veg. Sci.* 3, 157–164. doi: 10.2307/3235676

Kiær, L. P., Weisbach, A. N., and Weiner, J. (2013). Root and shoot competition: a meta-analysis. *J. Ecol.* 101, 1298–1312. doi: 10.1111/1365-2745.12129

Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., and Levine, J. M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* 29, 592–599. doi: 10.1111/1365-2435.12345

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Soft.* 82, 1–26. doi: 10.18637/jss.v082.i13

Lefcheck, J. S. (2016). piecewiseSEM: piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods Ecol. Evol.* 7, 573–579. doi: 10.1111/2041-210X.12512

Lewis, R. J., Szava-Kovats, R., and Pärtel, M. (2016). Estimating dark diversity and species pools: an empirical assessment of two methods. *Methods Ecol. Evol.* 7, 104–113. doi: 10.1111/2041-210X.12443

Lindborg, R., and Eriksson, O. (2004). Historical landscape connectivity affects present plant species diversity. *Ecology* 85, 1840–1845. doi: 10.1890/04-0367

Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I., et al. (2004). Rethinking plant community theory. *Oikos* 107, 433–438. doi: 10.1111/j.0030-1299.2004.13250.x

Luken, J. O. (1990). *Directing ecological succession*. *1st* London; New York: Chapman and Hall.

Martin, G., Courtial, A., Génin, A., Ramone, H., and Dutoit, T. (2022). Why grazing and soil matter for dry grassland diversity: new insights from multigroup structural equation modeling of micro-patterns. *Front. Ecol. Evol.* 10:879060. doi: 10.3389/fevo.2022.879060

Martinez-Almoyna, C., Saillard, A., Zinger, L., Lionnet, C., Arnoldi, C., Foulquier, A., et al. (2022). Differential effects of soil trophic networks on microbial decomposition activity in mountain ecosystems. *Soil Biol. Biochem.* 172:108771. doi: 10.1016/j.soilbio.2022.108771

McArdle, B. H., and Anderson, M. J. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82, 290–297. doi: 10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2

Michalet, R., Delerue, F., and Liancourt, P. (2023). Disentangling the effects of biomass and productivity in plant competition. *Ecology* 104:e3851. doi: 10.1002/ecy.3851

Münkemüller, T., Gallien, L., Pollock, L. J., Barros, C., Carboni, M., Chalmandrier, L., et al. (2020). Dos and don'ts when inferring assembly rules from diversity patterns. *Glob. Ecol. Biogeogr.* 29, 1212–1229. doi: 10.1111/geb.13098

Nerlekar, A. N., and Veldman, J. W. (2020). High plant diversity and slow assembly of old-growth grasslands. *Proc. Natl. Acad. Sci. U. S. A.* 117, 18550–18556. doi: 10.1073/pnas.1922266117

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., et al. (2022). *Vegan: community ecology package*. Available at: https://CRAN.R-project.org/package=vegan ()

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, 1–18.

Ossanna, L. Q. R., and Gornish, E. S. (2023). Efficacy of labile carbon addition to reduce fast-growing, invasive non-native plants: a review and meta-analysis. *J. Appl. Ecol.* 60, 218–228. doi: 10.1111/1365-2664.14324

Öster, M., Ask, K., Römermann, C., Tackenberg, O., and Eriksson, O. (2009). Plant colonization of ex-arable fields from adjacent species-rich grasslands: the importance of dispersal vs. recruitment ability. *Agric. Ecosyst. Environ.* 130, 93–99. doi: 10.1016/j.agee.2008.12.005

Palpurina, S., Chytrý, M., Hölzel, N., Tichý, L., Wagner, V., Horsák, M., et al. (2019). The type of nutrient limitation affects the plant species richness–productivity relationship: evidence from dry grasslands across Eurasia. *J. Ecol.* 107, 1038–1050. doi: 10.1111/1365-2745.13084

Pärtel, M., and Zobel, M. (1999). Small-scale plant species richness in calcareous grasslands determined by the species pool, community age and shoot density. *Ecography* 22, 153–159. doi: 10.1111/j.1600-0587.1999.tb00463.x

Prach, K., and Walker, L. R. (2011). Four opportunities for studies of ecological succession. *Trends Ecol. Evol.* 26, 119–123. doi: 10.1016/j.tree.2010.12.007

QGIS Development Team (2016). *QGIS geographic information system*. Available at: https://www.qgis.org

R Core Team (2022). *R: A language and environment for statistical computing*. Available at: https://www.R-project.org/

Rapson, G. L. (2023). Tertiary succession: a new concept to help vegetation restoration. *Restor. Ecol.* 31:e13683. doi: 10.1111/rec.13683

Römermann, C., Dutoit, T., Poschlod, P., and Buisson, E. (2005). Influence of former cultivation on the unique Mediterranean steppe of France and consequences for conservation management. *Biol. Conserv.* 121, 21–33. doi: 10.1016/j.biocon.2004.04.006

Saatkamp, A., Henry, F., and Dutoit, T. (2021). Romans shape today's vegetation and soils: two millennia of land-use legacy dynamics in Mediterranean grasslands. *Ecosystems* 24, 1268–1280. doi: 10.1007/s10021-020-00581-w

Schelfhout, S., De Schrijver, A., De Bolle, S., De Gelder, L., Demey, A., Du Pré, T., et al. (2015). Phosphorus mining for ecological restoration on former agricultural land. *Restor. Ecol.* 23, 842–851. doi: 10.1111/rec.12264

Seabloom, E. W., Borer, E. T., and Tilman, D. (2020). Grassland ecosystem recovery after soil disturbance depends on nutrient supply rate. *Ecol. Lett.* 23, 1756–1765. doi: 10.1111/ele.13591

Shipley, B. (2013). The AIC model selection method applied to path analytic models compared using a d-separation test. *Ecology* 94, 560–564. doi: 10.1890/12-0976.1

Smits, N. A. C., Willems, J. H., and Bobbink, R. (2008). Long-term after-effects of fertilisation on the restoration of calcareous grasslands. *Appl. Veg. Sci.* 11, 279–286. doi: 10.3170/2008-7-18417

Stoate, C., Báldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., et al. (2009). Ecological impacts of early 21st century agricultural change in Europe – a review. *J. Environ. Manag.* 91, 22–46. doi: 10.1016/j.jenvman.2009.07.005

Suding, K. N. (2011). Toward an era of restoration in ecology: successes, failures, and opportunities ahead. *Annu. Rev. Ecol. Evol. Syst.* 42, 465–487. doi: 10.1146/annurev-ecolsys-102710-145115

Sun, D., Mudrák, O., Ardestani, M. M., and Frouz, J. (2023). Unravelling the role of soil microflora from micro and macro aggregates in plant growth during primary and secondary successions. *Catena* 220:106655. doi: 10.1016/j.catena.2022.106655

Tansley, A. G., and Adamson, R. S. (1925). Studies of the vegetation of the English chalk: III. The chalk grasslands of Hampshire-Sussex border. *J. Ecol.* 13, 177–223. doi: 10.2307/2255283

Tomanek, G. W., Albertson, F. W., and Riegel, A. (1955). Natural revegetation on a field abandoned for thirty-three years in Central Kansas. *Ecology* 36, 407–412. doi: 10.2307/1929575

Török, P., Helm, A., Kiehl, K., Buisson, E., and Valkó, O. (2018). Beyond the species pool: modification of species dispersal, establishment, and assembly by habitat restoration. *Restor. Ecol.* 26, S65–S72. doi: 10.1111/rec.12825

Vellend, M. (2016). *The theory of ecological communities*. Princeton: Princeton University Press.

Walker, L. R., Walker, J., and Hobbs, R. J. (2007). *Linking restoration and ecological succession*. New York, NY: Springer.

Warton, D. I., Wright, S. T., and Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods Ecol. Evol.* 3, 89–101. doi: 10.1111/j.2041-210X.2011.00127.x

White, P. S., and Jentsch, A. (2004). “Disturbance, succession and community assembly in terrestrial plant communities” in *Assembly rules and restoration ecology: bridging the gap between theory and practice* (Washington, DC: Island Press), 342–346.

Wolff, A., Tatin, L., and Sauguet, F. (2015). La Réserve Naturelle des Coussouls de Crau. Plan de gestion 2015–2024. Section A: Diagnostic et vision. CEN-PACA, Saint-Martin de Crau, France. Available at: https://www.paca.developpement-durable.gouv.fr/IMG/pdf/1_plan_gestion_RNCC_2015-2024_section_A.pdf.

Yang, J., Su, P., Zhou, Z., Shi, R., and Ding, X. (2022). Environmental filtering rather than dispersal limitation dominated plant community assembly in the Zoige plateau. *Ecol. Evol.* 12:e9117. doi: 10.1002/ece3.9117

Yurkonis, K. A., and Meiners, S. J. (2004). Invasion impacts local species turnover in a successional system: invasion impacts local species turnover. *Ecol. Lett.* 7, 764–769. doi: 10.1111/j.1461-0248.2004.00636.x

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 01 June 2023
DOI 10.3389/fevo.2023.1152319

Check for updates

OPEN ACCESS

EDITED BY
Lilia L. Roa-Fuentes,
Pontifical Javierian University, Colombia

REVIEWED BY
Maan Rokaya,
Institute of Botany (ASCR), Czechia
Jeremy Goodall,
ARC Centre of Excellence for Electromaterials Science (ACES), Australia

*CORRESPONDENCE
Lucie Rivière
✉ lucie.rivere@uliege.be

RECEIVED 27 January 2023
ACCEPTED 17 May 2023
PUBLISHED 01 June 2023

CITATION
Rivière L, Sellier A, Dutoit T, Vidaller C, Buisson E and Mahy G (2023) The contribution of seedbank to the green roof plant community dynamics analogous to semi-natural grasslands.
Front. Ecol. Evol. 11:1152319.
doi: 10.3389/fevo.2023.1152319

The contribution of seedbank to the green roof plant community dynamics analogous to semi-natural grasslands

Lucie Rivière[1]*, Alizé Sellier[1], Thierry Dutoit[2], Christel Vidaller[2], Elise Buisson[2] and Grégory Mahy[1]

[1]Biodiversity and Landscape, TERRA Research Center, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, [2]Institut Méditerranéen de Biodiversité et d'Ecologie (IMBE), UMR Avignon University, CNRS, Aix-Marseille University, IRD, Avignon, France

Extensive green roofs have been shown to support native biodiversity and plant communities that are analogous to natural or semi-natural habitats such as grasslands. However, little is known about the role of soil seedbanks in the dynamic of extensive green roof plant communities. The purpose of this study was to analyze the seedbank that developed after 4 years of an extensive green roof analog to dry grassland plant community, seeded with 29 species. We aimed to understand the contribution of seedbank to the resilience of vegetation to harsh conditions of the roof and to colonization by surrounding spontaneous species. We monitored the plant species cover in 36 plots during 4 years in June (between 2018 and 2021), and sampled the seedbank in February 2021. Our results showed that the soil seedbank was dominated by transient spontaneous ruderals species, while the standing vegetation was still dominated by seeded grassland species. We found that seeded grassland species had poor seedbank stock, similar to their natural environments. The similarity index between the standing vegetation and the seedbank increased over time, and we measured a significant correlation between dominant species cover and their seedbank density. Spontaneous species cover was not correlated to the proportion of soil not colonized by seeded species cover, indicating that gaps in vegetation did not influence the development of spontaneous species. Our findings highlight the importance of seedbank in the dynamic of green roof vegetation and demonstrate that analogous habitat species exhibit similar behavior as in their natural environments.

KEYWORDS

plant dynamic, ruderal, spontaneous species, green infrastructure, seed persistence, urban ecology, substrate depth, sun exposure

1. Introduction

Extensive green roofs (EGR) are a type of urban ecosystem that offer a range of ecosystem services (Oberndorfer et al., 2007; Kowarik, 2011). One of the ecosystem services provided by EGRs is the support of urban native biodiversity, through the implementation of diversified native plant communities based on the analogous habitat hypothesis

(Lundholm and Richardson, 2010; Rivière et al., 2022). The analogous habitats hypothesis proposes that species which are adapted to similar abiotic conditions in natural habitats can also be successful on green roofs (Lundholm, 2006) and can serve as a starting point for selecting species. Green roofs provide solutions to develop urban grassland and herbaceous ecosystem networks in cities (Sutton et al., 2012; Williams et al., 2014; Thuring and Grant, 2015). However, harsh environmental conditions on extensive green roofs (e.g., high level of solar radiation, shallow substrate, poor water retention capacity) associated with future climate evolution can threaten the success of these native plant communities, and associated ecosystem services, when the functioning and dynamic of EGR ecosystems are not well-understood.

Green roof plant communities are dynamic. When initiated by seeding on bare substrate, the EGR plant community is marked by changes in plant composition over time as this situation relates to primary ecological succession conditions (Brown and Lundholm, 2015). The seeded plant community evolves through time in accordance to seeded species response to environmental conditions at the roof scale: roof height, slope, exposure, substrate depth and composition (Madre et al., 2014; Brown and Lundholm, 2015). EGR are also characterized by local microenvironmental variations impacting the composition and dynamic of the plant community (Heim and Lundholm, 2014; Roulston et al., 2020; Bradbury, 2021; Rivière et al., 2022). In addition to the response of seeded species to environmental conditions, a key driver of EGR plant community evolution is spontaneous plant colonization with non-seeded plant species (Thuring and Dunnett, 2019; Vanstockem et al., 2019). Weedy or unwanted species colonizing spontaneously EGR are identified as a limit to EGR acceptance and maintenance (Vanstockem et al., 2019). Spontaneous colonization by plant species is often linked to presence of bare soil or gaps in the vegetation cover due to the extreme abiotic growing conditions on EGR (Köhler, 2006; Sutton and Lambrinos, 2015).

Soil seedbank is a key component of plant community dynamics (Thompson et al., 1997; Vandvik et al., 2016). It influences the vegetation dynamic in the short and long term (Basto et al., 2018). The seedbank can provide information on species that may have formerly existed in the vegetation, species that represent the pool for the vegetation succession, and, represents the source of regenerative potential for the restoration of altered plant communities. Seedbanks on EGR [being biodiversity reservoirs (Vandvik et al., 2016)] may be crucial as an insurance for maintaining plant communities during periods of extreme conditions (e.g., extreme drought or frost) and can potentially offer a basis for restoration for vegetation gaps. Despite its importance in understanding vegetation response to extreme EGR conditions, the study of seedbank from extensive green roof (Vanstockem et al., 2018) or even urban areas (Albrecht et al., 2011) has received little attention. In an explorative study of seedbanks on 109 EGRs in temperate Europe, Vanstockem et al. (2018) confirm that EGRs do develop seedbanks, dominated by weedy plant species and exhibiting a strong discrepancy with vegetation composition.

To promote native biodiversity, EGR should be considered analogous to natural habitats (Lundholm, 2006). The harsh environmental constraints of EGR are similar to abiotic conditions encountered on species rich dry grasslands and rocky environments in temperate Western Europe (high solar radiation, low soil depth (<20 cm) and high variability in soil water retention capacity). Western Europeans dry grasslands are composed of isolated climax herbaceous habitats on most harsher environmental conditions and, for the largest surfaces, seral semi-natural habitats originating from centuries of extensive grazing and mowing. Rivière et al. (2022), demonstrated experimentally that extensive green roofs can act as analogous habitats for native flora of European dry calcareous grasslands (71% of native seeded grassland species established on an experimental green roof). The most successful species were common dry grassland species such as grass species, succulent species (*sedum sp.*), *Echium vulgare* L. and *Papaver argemone* L. However, success of native grassland plant species, plant community composition and structure were demonstrated to vary through time and to depend on microenvironmental conditions at the roof scale, with large surface of bare soils left after 3 years in the most constrained conditions (low substrate depth and high solar exposition). Decrease in plant cover was also measured in years with severe drought. The contribution of spontaneous ruderal species to the plant communities increased over time. Studies of seedbank in natural situations have shown that calcareous grassland species are mainly perennial stress tolerant species presenting mostly transient seedbank that stay viable in the soil for a very limited period of time and/or are absent from the seedbank (Dutoit and Alard, 1996; Willems and Bik, 1998; Bisteau and Mahy, 2005). A lack of well-developed permanent seedbank of target seeded species can be a threat for long term maintenance of analogous dry grassland vegetation of EGR facing colonization by spontaneous ruderal species.

In this study, we analyzed the seedbank contribution to the plant community of an extensive green roof analog to dry calcareous grassland after 4 years of development in order to understand the potential contribution of seedbank to the resilience of vegetation to harsh EGR conditions and to colonization by spontaneous species. The specific questions asked are: (i) what are the main seedbank characteristics and composition of an analogous urban grassland developed on an extensive green roof, (ii) what is the level of similarity between seedbank and vegetation, (iii) how is the seedbank and abundance of spontaneous species related to vegetation gap and microenvironmental variations on EGR.

2. Materials and methods

2.1. Study site and experimental design

The studied green roof is located on top of the TERRA Research Center of Gembloux Agro-Bio-Tech, Belgium (50°33′48′′ N 4°41′52′′ E). According to Köppen (1923) climate classification, the climate of the region is oceanic temperate (Cfb) (Service Fédéral Belge, 2019).

In October 2017, the EGR was installed with a commercial substrate for EGR: lightweight substrate Zinco© based on a mix of recycled tiles, bricks and ceramic. The roof included nine parcels ranging in size from 14 to 57 m^2 with two substrate depths (6 and 11 cm) and an East-West orientation. The roof is bordered on three sides by walls (North, East, and South), resulting in global disparities in sun exposure across parcels. Skylights installed between parcels create an additional fine-scale shade gradient

(**Figure 1**). Three classes of sun exposure (maximum sun exposure on a sunny summer day) were defined: low (3–6 h), Medium (6–9 h), and High (9–12 h). The two parameters of substrate depth and sun exposure were noted: D1 (6 cm depth), D2 (11 cm depth), S1 (low sun exposure), S2 (medium sun exposure), and S3 (high sun exposure) (Rivière et al., 2022).

A mix of 29 native species from Belgian dry calcareous grasslands were selected based on their presence along the soil depth gradient in Belgian dry grasslands (Piqueray et al., 2007; **Table 1**). Calcareous grasslands are not present in the ecological landscape surrounding the experimental green roof but 12 seeded species have been recorded in 2 km^2 around it (Delescaille and Delaite, 2011). Three native Belgian *Sedum* species were chosen because they are succulent plants already commonly used on ExGR and adapted to harsh conditions (Butler and Orians, 2009). The species were seeded uniformly across the parcels on 10th November 2017, 830 seeds/species/m^2 for grass species and 110 seeds/species/m^2 for other species. During the research period (2017–2021), no overseeding, irrigation, fertilizers or mowing were applied. Rivière et al. (2022) studied the vegetation development according to substrate depth, sun exposure and changes over time, and showed a strong effect of time since sowing associated with the effects of these microenvironmental variation on plant community composition, plant cover and specific richness.

2.2. Data collection

Thirty-six permanent 1 m^2 plots were installed on the nine parcels reflecting the variation of substrate depth and sun exposure (**Figure 1**). Plant species covers were visually measured in these 36 plots in June 2018, 2019, 2020 and 2021, at the peak of vegetative development (Rivière et al., 2022).

The seedbank was sampled in February 2021, before spring germination in order to collect seeds of both transient and persistent seedbanks under Atlantic and Continental climates, following Bekker et al. (1998). In each of the 36 plots, a sample of 10 bulked soil cores (2.5 cm diameter) was collected. Cores were divided in two layers for D1 6 cm deep parcels (layer 1: 0–3 cm and layer 2: 3–6 cm) and three layers for D2 11cm deep parcels (layer 1: 0–3 cm, layer 2: 3–6 cm and layer 3: 6–11 cm). A total of 88 samples were obtained. To extract the seedbank, the dried samples were sieved at 3.66 mm in order to remove roots, vegetative parts and stones. The sieved fractions were sown in 40 cm × 28 cm × 6.5 cm seedling trays with a 3 cm Argex marble and 1 cm organic matter (La plaine Chassart©). The trays were placed in homogeneous greenhouse conditions (22°C, 76% RH) and watered regularly by capillarity. The number of seedlings per species in each tray was counted at weekly intervals and removed after identification; monitoring continued until germination ceased after 15 weeks. The seedlings of the five seeded Poaceae seedlings could not be distinguished and were classified as “grass sp.”

LEDA database (Kleyer et al., 2008) was used to determine seed mass and seed lifetime index (SLI). For seed mass, only the mean “one-seeded generative dispersule” was considered per species. A mean SLI was calculated per species based on their persistence in the seedbank: 0 (all data are transient), 1 (all data are persistent). Seedbank can be transient if the seeds are present in the seedbank less than 1 year; short-term persistent between 1 and 5 years; or long-term persistent more than 5 years. In general, long-term seeds are found in both the deepest and shallowest levels of the substrate, whereas short-term seeds are exclusively found in the shallower layer of the substrate (Christoffoleti and Caetano, 1998).

The status of each species was determined by whether they were seeded (seeded, sd) or not (spontaneous, sp). Spontaneous species represent all unseeded species, annual or perennial, native or alien. The taxonomic reference was the “Nouvelle flore de la Belgique” (Lambinon et al., 2012). Species were classified in three ecological groups according to their natural habitat: grassland species, ruderal species and forest species (Lambinon et al., 2012; **Table 1**).

2.3. Data analyses

Similarity in species composition (presence/absence) between the initial seeding mix, species composition in vegetation in 2018–2021 and species composition in the 2021 seedbank was assessed with (1) the similarity index of Sokal and Michener (Perrier et al., 2003), and, (2) a Principal Component Analysis (PCA) (“princomp,” stats) performed on all plots. For each species, a Pearson correlation was calculated (“cor,” stats) to identify species that correlated most with the axes of the PCA.

The relationship between the most abundant species in the vegetation and the most abundant species in the seedbank (found in at least half of the plots) was determined by Pearson correlation tests (“cor,” stats) to establish if species cover was correlated to its seedbank density. To test if grassland vegetation development influenced establishment of spontaneous species by pre-empting bare soils surfaces (Vanstockem et al., 2018), we tested the regression between the spontaneous species total cover and the proportion of soil not colonized by grassland species in 2021 (100%–%total seeded species cover in 2021) with a linear regression (“lm,” cor).

To test whether microenvironmental condition (depths: D1, D2 and sun exposure: S1, S2, S3) variations on the experimental EGR influenced similarly plant community and seedbank variations, we performed independent Principal Coordinate Analysis (PCoA) on (1) cover of species in the vegetation, (2) seed density of species in seedbank, in all plots. Ordispider were used to depict the relationships between microenvironmental conditions and position of plots in the factorial space.

Data analyses were performed in R 4.0.2 (R Core Team, 2022).

3. Results

3.1. Seedbank characteristics

In 2021, 4 years after the creation of the green roof, the seedbank included 18 species (nine seeded species and nine spontaneous species, grass species considered as a single taxon due to difficulties identify seedlings) with an average of 2.5 ± 1.1 species and 120 ± 172 seeds per square meter plot. There was no seedbank observed in three plots. Seed density decreased with substrate depth with 42.8% of seed located at 0–3 cm depth, 34.7% at the 3–6 cm depth, and 22.5% at the 6–11 cm (**Figure 2**).

FIGURE 1
The studied extensive green roof and the permanent plots distribution.

The standing vegetation included 29 species (16 seeded species and 13 spontaneous species, grass species considered as one taxon) with an average of 5.7 ± 1.8 species and 47.3% ± 39.3% of cover per square meter plot (**Figure 2**). There was no plot without vegetation cover.

The seedbank (mean SLI: 0.34 ± 0.13) and the vegetation (mean SLI: 0.25 ± 0.16) were both composed of species with a transient seedbank. All species have a SLI of less than 0.5 except *Chenopodium album* L. (0.59) and *Portulaca oleracea* L. (0.68) both spontaneous species and present in the vegetation and in the seedbank.

3.2. Seedbank and vegetation similarity

The seedbank was dominated by seeds from spontaneous species (84.8%), while seeds from seeded species represent only 15.23%. In contrast, seeded species represent 70.4% of plant cover in the vegetation, while spontaneous species represented only 29.6%. The seedbank was dominated by the ruderal ecological group (84.4% of all seeds) followed by grassland ecological group (15.2%). In contrast, the plant cover was dominated by species from the grassland ecological group (82.6%), followed by species from ruderal ecological groups species (17.0%). Both the seedbank and the standing vegetation had low occurrence of species from forest ecological group (0.34 and 0.40%, respectively).

The seedbank was dominated at 65.6% of *P. oleracea*. While spontaneous species dominated the seedbank (*P. oleracea*, *C.album* and *Senecio vulgaris* L. at 65.5, 9.3, and 5.9%, respectively), they were seldom developed in the vegetation with cover less than 1% per species, except for *P. oleracea* (7.56%/m^2) and *Trifolium arvense* L. (5.14%/m^2), a cover similar to dominant seeded species (**Figure 2**).

Only nine out of 24 seeded species (considering all grasses as a single taxon) were detected in the seedbank with densities ranging from 6.7 to 254.4 seeds/m^2, from which eight were also present in the vegetation. Grasses (254.4 seeds/m^2, 9.08% cover), *Sedum acre* L. (160.7 seeds/m^2, 2.08% cover), *Silene vulgaris* (Moench) Garcke (87.03 seeds/m^2, 0.36% cover), *Sedum album* L. (53.6 seeds/m^2, 3.9% cover), and *Echium vulgare* L. (20.08 seeds/m^2, 5.03% cover) were the seeded species developing the densest seedbank. Only *S. acre* has a higher seedbank density than the initial seeding mix (110 seeds/m^2). Eight seeded species present in the vegetation were not detected in the seedbank, including *Anthyllis vulneraria* L., one of the vegetation dominant species. Seven seeded species were not observed in the vegetation, nor in the seedbank (**Figure 2**).

The lowest similarity (Sokal and Michener index) in species composition was measured between the initial seeding mix and the seedbank after 4 years of vegetation development. Similarity between initial seeding mix composition and standing vegetation decreased over time from 0.75 to 0.50 while similarity between the vegetation and the seedbank increased over time from 0.5 to 0.61 (**Figure 3**). The vegetation differed each year a little more from the initial seeding and became more similar to the seedbank sampled in 2021.

The first and second axes of the PCA (**Figure 4**) based on the presence/absence of species in the initial seeding mix, the plant community in 1 m^2 plots in 2018, 2019, 2020, and 2021 and the seedbank in 1 m^2 plots in 2021 explained 61.8% of the global species composition variation (Comp1 = 54.1%–eigenvalue 1 = 2.7, Comp2 = 7.7%–eigenvalue 2 = 0.4). Axis 1 strongly differentiated the initial seeding mix from standing vegetation and seedbank. Axis 2 indicated a trend for plant community to become more similar to seedbank over years. The correlation circle indicated that the first axis was correlated with the presence of seeded species. The plant community of 2018, 2019, 2020, and 2021 and the seedbank were differentiated from the initial seeded community with the presence of spontaneous species and with the absence of seeded species that never germinated over time (*Hieracium pilosella* L., *Leucanthemum vulgare* Lam., *Hypochaeris radicata* L., *Primula veris* L., *Thymus pulegioides* L., and *Tragopogon pratensis* L.).

TABLE 1 Seeded or spontaneous species observed in 2018, 2019, 2020, 2021 or in the seedbank, the code used in figures and their status.

Species	Code	Status	Ecological group
Anthyllis vulneraria L.	Ant_vul	Seeded (sd)	Grassland species
Centaurea scabiosa L.	Cen_sca	Seeded (sd)	Grassland species
Daucus carota L.	Dau_car	Seeded (sd)	Grassland species
Dianthus carthusianorum L.	Dia_car	Seeded (sd)	Grassland species
Echium vulgare L.	Ech_vul	Seeded (sd)	Grassland species
Gram sp.	Gram_sp	Seeded (sd)	Grassland species
Hieracium pilosella L.	Hie_pil	Seeded (sd)	Grassland species
Hypochaeris radicata L.	hyp_rad	Seeded (sd)	Grassland species
Leucanthemum vulgare Lam.	Leu_vul	Seeded (sd)	Grassland species
Lotus corniculatus L.	Lot_cor	Seeded (sd)	Grassland species
Medicago lupulina L.	Med_lup	Seeded (sd)	Grassland species
Papaver argemone L.	Pap_arg	Seeded (sd)	Grassland species
Primula veris L.	Pri_ver	Seeded (sd)	Grassland species
Rhinanthus minor L.	Rhi_min	Seeded (sd)	Grassland species
Rumex acetosella L.	Rum_ace	Seeded (sd)	Grassland species
Sanguisorba minor Scop.	San_min	Seeded (sd)	Grassland species
Scabiosa columbaria L.	Sca_col	Seeded (sd)	Grassland species
Sedum acre L.	Sed_acr	Seeded (sd)	Grassland species
Sedum album L.	Sed_alb	Seeded (sd)	Grassland species
Sedum rupestre L.	Sed_rup	Seeded (sd)	Grassland species
Silene vulgaris (Moench) Garcke	Sil_vul	Seeded (sd)	Grassland species
Thymus pulegioides L.	Thy_pul	Seeded (sd)	Grassland species
Tragopogon pratensis L.	Tra_pra	Seeded (sd)	Grassland species
Verbascum sp.	Ver_sp	Seeded (sd)	Grassland species
Acer sp.	Ace_sp	Spontaneous (sp)	Forest species
Betula sp.	Bet_sp	Spontaneous (sp)	Forest species
Buddleja davidii Franch.	Bud_dav	Spontaneous (sp)	Ruderal species
Chenopodium album L.	Che_alb	Spontaneous (sp)	Ruderal species
Cirsium vulgare (Savi) Ten.	Cir_vul	Spontaneous (sp)	Ruderal species
Epilobium tetragonum L.	Epi_tet	Spontaneous (sp)	Ruderal species
Geranium sp.	Ger_sp	Spontaneous (sp)	Ruderal species
Lactuca serriola L.	Lac_vir	Spontaneous (sp)	Ruderal species
Portulaca oleracea L.	Por_ole	Spontaneous (sp)	Ruderal species
Senecio vulgaris L.	Sen_vul	Spontaneous (sp)	Ruderal species
Sonchus asper (L.) Hill	Son_asp	Spontaneous (sp)	Ruderal species
Sonchus oleraceus L.	Son_ole	Spontaneous (sp)	Ruderal species
Trifolium arvense L.	Tri_arv	Spontaneous (sp)	Grassland species
Trifolium pratense L.	Tri_pra	Spontaneous (sp)	Grassland species
Vicia sativa L.	Vic_sat	Spontaneous (sp)	Grassland species

3.3. Influence of plant communities on seedbank

Correlation between plant cover and seed density at 1 m^2 plot scale was tested for species found in the seedbank and vegetation cover in at least half of plots. Pearson correlation tests indicated a significant correlation and a high correlation for grass species ($P < 0.001$–cor = 0.31) and *P. oleracea* ($P < 0.001$–cor = 0.57). The Pearson correlation test was high with an almost significant P value for *E. vulgare* ($P = 0.07$—cor = 0.32), *S. acre* ($P = 0.07$—cor = 0.75), and *C. album* ($P = 0.08$—cor = 0.34). The seedbank density and the vegetation cover of *S. vulgaris* were not significantly correlated ($P = 0.50$—cor = −0.12).

The proportion of soil not colonized by seeded species (100%–%total seeded species cover in 2021) had no significant effect on the spontaneous species cover (linear regression, $P = 0.93$, $R^2 = -0.03$).

3.4. Influence of microenvironmental variation on plant communities and seedbank

When examining the effect of microenvironmental variation on plant community composition of standing vegetation in 2021

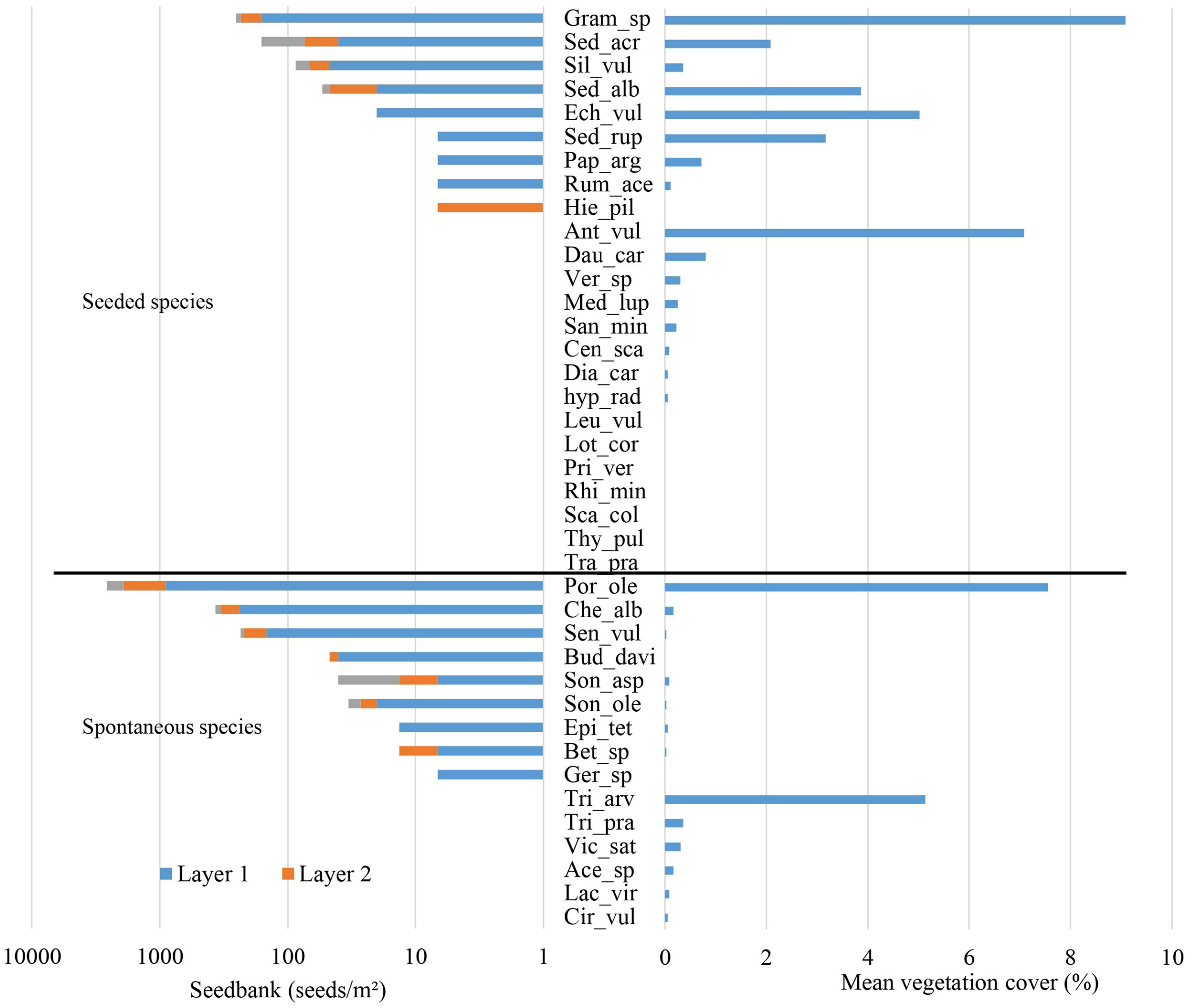


FIGURE 2
Seedbank density (seeds/m^2) and mean vegetation cover of 2021 (%) for seeded species and spontaneous species (see **Table 1** for species name).

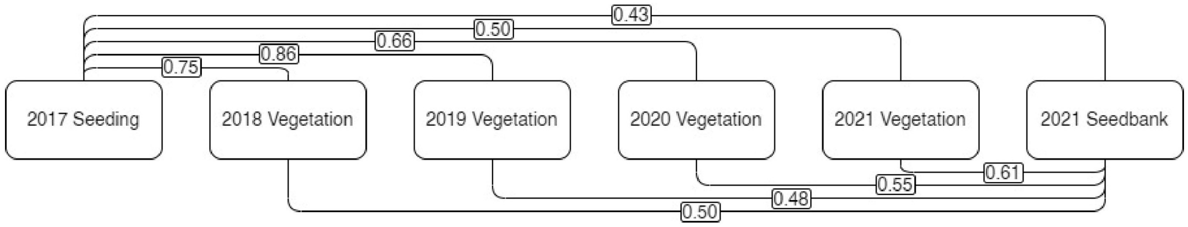


FIGURE 3
Sokal and Michener index between 2017 seeding, 2018, 2019, 2020, and 2021 vegetation cover and 2021 seedbank.

(taking into account plant species cover), the first and second axes of the PCoA (**Figure 5A** explained 25.2% of the global variation (Dim1 = 13.7%–eigenvalue = 1.9, Dim2 = 11.5%–eigenvalue = 1.6). Position of ordispider gravity centre on first axis discriminated plant communities in plots with shallow substrate depth (D1: 6 cm) (negative coordinates) from plant communities on deeper substrate (D2: 12 cm) (positive coordinates). Position of ordispider gravity centre on second axis discriminated plant communities in plots with high sun exposure S3 (negative coordinates) from plant communities with medium to low sun exposure S1 and S2 (positive coordinates).

When examining the effect of microenvironmental variation on seedbank composition (taking into account species seed density), the first and second axes of the PCoA (**Figure 5B** explained 27.1% of the global variation (Dim1 = 14.4%–eigenvalue = 2.0, Dim2 = 12.7%–eigenvalue = 1.7). Position of ordispider gravity centre on first axis discriminated seedbank composition in plots with high sun exposure S3 (positive coordinates), characterized

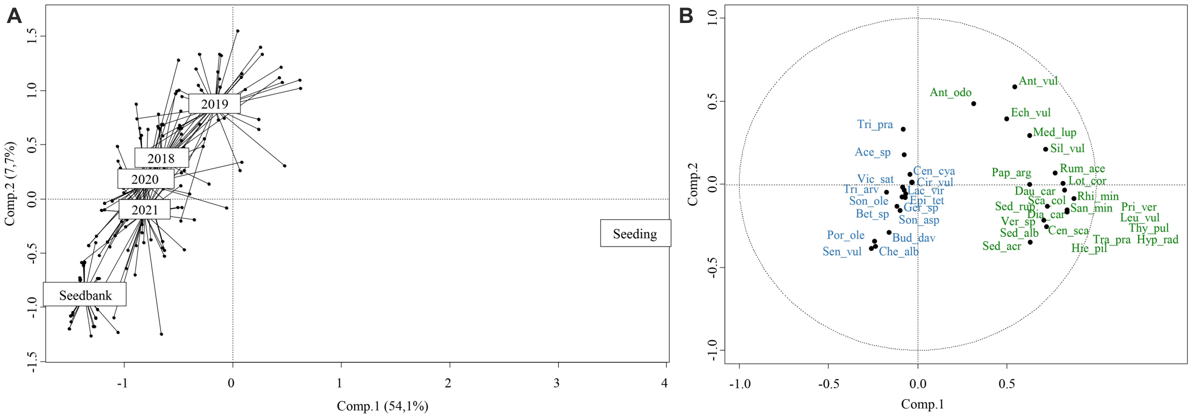


FIGURE 4

Principal Component Analysis (PCA) based on presence/absence species composition for initial seeding, 2018, 2019, 2020, and 2021 vegetation cover and 2021 seedbank. **(A)** Ordi spiders pool by year. **(B)** Correlation circle of species, blue species: spontaneous species, green species: seeded species.

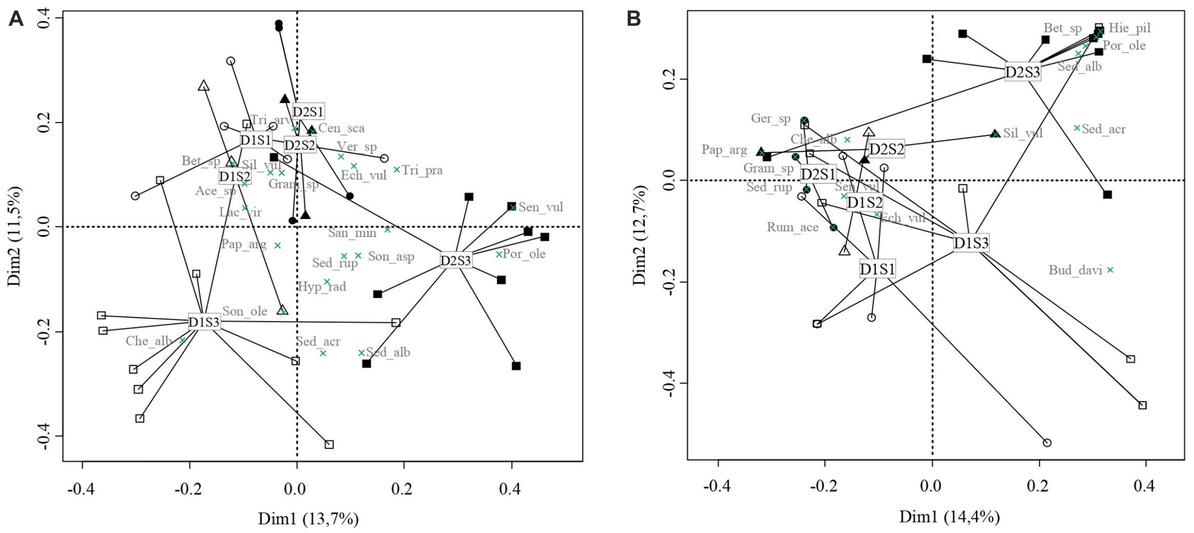


FIGURE 5

Principal Coordinate Analysis (PCoA) based on **(A)** plant composition of standing vegetation in 2021 and on **(B)** seedbank composition. Ordi spiders are pooled according to microenvironmental conditions. Symbols represent sun exposure modalities (circle = S1: low sun exposure, triangle = S2: medium sun exposure, and square = S3: high sun exposure) and substrate depth modalities (white = D1: shallow depth 6 cm, black = D2: deep depth 11 cm). Species are indicated with green crosses.

by the presence of *P. oleracea*, *Buddleja davidii* Franch., and *Betula* sp, from seedbank composition in plots with medium to low sun exposure S1 and S2 (negative coordinates), characterized by the presence of *C. album*, *Geranium* sp. and grass sp. Position of ordispider gravity centre on second axis discriminated seedbank composition in plots with shallow substrate depth (D1: 6 cm) (negative coordinates) from seedbank composition on deep substrate depth (D2: 12 cm) (positive coordinates).

4. Discussion

Increasing attention has been paid to plant community dynamics on extensive green roof in ecological literature (Roulston et al., 2020; Bradbury, 2021; Rivière et al., 2022). Change in plant community over time is key to EGR design and their acceptability (Thuring and Dunnett, 2019; Vanstockem et al., 2019). It is also a major determinant of EGR capacity to sustain native biodiversity through development of analog habitat (Lundholm, 2006; Rivière et al., 2022). In this study, we examined the development of seedbank in relation to the installation of an analogous dry grassland on an experimental EGR. In contrast to our recent knowledge increase on above ground vegetation patterns and processes on green roofs (Madre et al., 2014; Brown and Lundholm, 2015), seedbank development and its role for green roof vegetation has been seldom explored directly (Vanstockem et al., 2018). The study of seedbank is important to understand its contribution to the resilience of vegetation to harsh EGR conditions. Specifically, a lack of well-developed permanent seedbank of target seeded species can be a threat for long term maintenance of analogous dry grassland vegetation of EGR facing colonization by spontaneous ruderal species.

Our study of the standing plant community evolution on an experimental EGR indicates that green roofs work as an analogous habitat supporting seeded species from native dry grasslands (Rivière et al., 2022). However, the evolution over time of plant community on the EGR diverges from the initial seeding by the absence of a set of seeded grassland species and the increase in spontaneous species cover over time. The species richness of spontaneous species tends to increase to the same level as species richness of seeded species (12 *vs.* 16) in 4 years. Development of a high richness of spontaneous species on experimental green roofs over a short time period has been repeatedly reported. For example, Dunnett et al. (2008) in Sheffield, UK, identified 35 colonizing species for 15 planted species, Olly et al. (2011) on experimental plots in Birmingham, UK, identified 29 spontaneous species for 17 seeded species and Bates et al. (2013) identified 31 and 37 colonizing species on two brown roofs in the same city. All the spontaneous species observed on the studied EGR are anemochorous except *C. album*, *Geranium* sp, *P. oleracea* and *Vicia sativa* L. Kleyer et al. (2008). Those latter species may have been imported to the roof by birds or by people visiting the roof. This suggests that most spontaneous species reach our EGR by wind, a pattern reported by Madre et al. (2014). We cannot exclude that some species might have been introduced *via* seed-contaminated substrate, however, we think that this is negligible as there was very low spontaneous species development on the first year of the experience.

Using a "space for time" approach, Vanstockem et al. (2018) demonstrated the presence of seedbanks on established extensive green roofs aged of 19 years. In the current study, for the first time, we experimentally demonstrate the accumulation of a seedbank on an experimental green roof over a short period of time (4 years) from its settlement from bare substrate. A total of 18 species were found in the seedbank. Mean seedbank SLI indicated a transient global seedbank, but seed accumulation in the deepest substrate layers suggested ongoing constitution of a permanent seedbank, mainly due to spontaneous species with permanent seedbanks, such as *C. album* and *P. oleracea.*

Despite a set of common species between the seedbank and the vegetation, and a trend for plant community to become more similar to the seedbank through time, in depth comparison reveal divergence between both, with spontaneous ruderal species dominating the seedbank (mainly *P. oleracea*) when considering seedbank density, and seeded grassland species dominating the vegetation when considering the species cover. High dissimilarity between the composition of species of the seedbank and the vegetation has been measured in established EGR and the majority of species in EGR seedbanks are spontaneous colonizers (Vanstockem et al., 2018).

Only a small proportion (9 out of 24) of seeded grassland species are detected in the seedbank with mostly lower seed densities as compared to the original seeding, indicating that a seed reserve has not accumulated over time. Limited development of grassland seedbank is due to the non-development of some seeded species (eight species) and to the absence of seedbank for a set of species (eight) present in the vegetation.

Comparison with the seedbank of (semi-)natural systems with comparable environmental characteristics [habitat template *sensu* Lundholm (2006)], such as calcareous grasslands on shallow soils, can provide useful insights on EGR seedbanks. Many authors showed that species dominating calcareous grassland vegetation were mostly absent from the seedbank, and when present were not abundant (Davies and Waite, 1998; Bisteau and Mahy, 2005; Bossuyt et al., 2006). Almost all calcareous grassland species produce short-lived (transient) seeds that stay viable in the soil for a limited time period. Grassland species invest more resources in individual survival than in seeds production (Eriksson and Eriksson, 1997).

Spontaneous species observed on the experimental EGR are mainly ruderal and for the majority (60%) have accumulated a seedbank despite low cover in the above ground vegetation, to the exception of *P. oleracea,* which was one of the dominant species of the vegetation. Vanstockem et al. (2018) also reported a dominance of ruderal species in seedbank of established EGR. Ruderal species allocate more resources to producing a large number of seeds rather than developing their cover (Grime, 1988; Šerá and Šerý, 2004). Ruderal spontaneous species are also present in seedbank composition of natural dry grassland but have a limited development in the vegetation (Bisteau and Mahy, 2005). In contrast to Vanstockem et al. (2018) who observed limited presence in the seedbank species pool in comparison to the vegetation, the two most abundant phanerophyte species in the seedbank of our EGR (*Buddleja davidii* Franch., *Betula* sp) were almost absent from the vegetation, indicating that seed rain may be an agent responsible for seed accumulation in the seedbank (Davies and Waite, 1998). The presence of *B. davidii* in the seed bank may pose a potential problem for future vegetation development on the roof as it has a very high invasive potential in disturbed, open and dry environments (Tallent-Halsell and Watt, 2009; Monty et al., 2019), even though it is currently absent from the established vegetation in permanent plots.

We demonstrate that after 4 years of development, both standing vegetation and seedbank species composition were influenced by the variation of environmental factors: substrate depth and sun exposure. The substrate depth is known to be an important factor structuring green roof vegetation both for seeded/planted species and for colonizing species (Durhman et al., 2007; Köhler and Poll, 2010; Madre et al., 2014). In more stressful environment on our EGR (low substrate depth, high sun exposure) vegetation cover was limited with large proportion of bare substrate (Rivière et al., 2022). Vegetation gaps are an important component of seeded EGR, during the vegetation development phase and along the life-cycle of EGR when extreme environmental conditions and abiotic conditions can locally affect vegetation (Vanstockem et al., 2019). Colonizing plants can develop on green roofs because there is available space due to changes in the planted populations, with a decrease in some of the planted species (Dunnett et al., 2008). Gaps in vegetation can impact seedbank characteristics and the development of vegetation by providing an opportunity for colonization by spontaneous and ruderal species (Vanstockem et al., 2018). However, in our study, we do not find a relationship between space left vacant by grassland vegetation and spontaneous species development. This can result from extreme environmental conditions affecting in the same way the two categories of species and preventing ruderal spontaneous species to germinate and colonize bare substrate. This lack of gap colonization potential may be reinforced by the fact that seedbank density was correlated to dominant species cover in the vegetation, indicating, that seedbank accumulates in the vicinity of mature individuals growing in the most favorable environment of the green roof. Also, the role of the seedbank in gap regeneration depends on the severity of disturbance which should relocated buried seed to the surface. In undisturbed vegetation, seedlings very rarely originate from the seedbank (Milberg and Persson, 1994; Kalamees and Zobel, 2002).

In conclusion, in contrast to Vandvik et al. (2016) and Vanstockem et al. (2019) suggestion that seedbanks on EGR could serve as a diversity insurance during periods of extreme conditions (e.g., extreme drought or frost) and can therefore potentially offer a basis for restoration of vegetation gaps, we experimentally measured that the maintenance of a diversified analogous grassland on an extensive green roof cannot rely mainly on an accumulated seedbank. Grassland seeded species did not contribute significantly to the seedbank. Seeded community is therefore unstable and vulnerable to sudden and profound changes under extreme hydric stress conditions. In this case, standing vegetation will be dominated by spontaneous species and the habitat developed will no longer be analogous to dry grassland. Maintenance of target grassland vegetation, in response to disturbance by extreme events, will depend mainly on the perennial habit of grassland species and their high tolerance to stress. Punctual seeding intervention may be needed in case of extreme climatic events affecting grassland species survival.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GM and LR carried out the conception, design of the study, and wrote the first draft of the manuscript. LR and AS carried out the experiment and performed the statistical analysis. All authors contributed to the conception of the manuscript, manuscript revision, read, and approved the submitted version.

Funding

This work was funded by ARC CityRoof (University of Liège).

Acknowledgments

The authors acknowledge the ARC CityRoof project for funding and UCL for providing access to their greenhouses.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Albrecht, H., Elisabeth, E., Thomas, L., and Clara, T. (2011). The soil seed bank and its relationship to the established vegetation in urban wastelands. *Landsc. Urban Plan.* 100, 87–97. doi: 10.1016/j.landurbplan.2010.11.011

Basto, S., Thompson, K., Grime, J., Fridley, J., Calhim, S., Askew, A., et al. (2018). Severe effects of long-term drought on calcareous grassland seed banks. *NPJ Clim. Atmos. Sci.* 1:1. doi: 10.1038/s41612-017-0007-3

Bates, A., Sadler, J. P., and Mackay, R. (2013). Vegetation development over four years on two green roofs in the UK. *Urban For. Urban Green.* 12, 98–108. doi: 10.1016/j.ufug.2012.12.003

Bekker, R. M., Bakker, J. P., Grandin, U., Kalamees, R., Milberg, P., Poschlod, P., et al. (1998). Seed size, shape and vertical distribution in the soil: Indicators of seed longevity. *Funct. Ecol.* 12, 834–842. doi: 10.1046/j.1365-2435.1998.00252.x

Bisteau, E., and Mahy, G. (2005). Vegetation and seed bank in a calcareous grassland restored from a *Pinus* forest. *Appl. Veg. Sci.* 8, 167–174. doi: 10.1658/1402-2001

Bossuyt, B., Butaye, J., and Honnay, O. (2006). Seed bank composition of open and overgrown calcareous grassland soils—a case study from southern Belgium. *J. Environ. Manag.* 79, 364–371. doi: 10.1016/j.jenvman.2005.08.005

Bradbury, C. (2021). *Functional difference in plant communities as a driver of green roof ecosystem services.* Ph.D. thesis. Halifax, NS: Saint Mary University. Available online at: http://library2.smu.ca/xmlui/handle/01/29518

Brown, C., and Lundholm, J. (2015). Microclimate and substrate depth influence green roof plant community dynamics. *Landsc. Urban Plann.* 143, 134–142. doi: 10.1016/j.landurbplan.2015.07.009

Butler, C., and Orians, C. (2009). Session 3.3: Natives vs. non-natives, the debate on the merits of each continues. *Conf. Proc.* 1–14.

Christoffoleti, P. J., and Caetano, R. S. X. (1998). Soil seed banks. *Sci. Agric.* 55, 74–78. doi: 10.1590/S0103-90161998000500013

Davies, A., and Waite, S. (1998). The persistence of calcerous grassland species in the soil seed bank under developing and established scrub. *Plant Ecol.* 136, 27–39. doi: 10.1023/A:1009759227900

Delescaille, L.-M., and Delaite, S. (2011). Latlas floristique de wallonie: Où en est-on? *Adoxa* 68, 17–19.

Dunnett, N., Nagase, A., and Hallam, A. (2008). The dynamics of planted and colonising species on a green roof over six growing seasons 2001-2006: Influence of substrate depth. *Urban Ecosyst.* 11, 373–384. doi: 10.1007/s11252-007-0042-7

Durhman, A., Bradley Rowe, D., and Rugh, C. L. (2007). Effect of substrate depth on initial growth, coverage, and survival of 25 succulent green roof plant taxa. *HortScience* 42, 588–595. doi: 10.21273/HORTSCI.42.3.588

Dutoit, T., and Alard, D. (1996). "Chapitre second. Mécanisme d'une succession végétale secondaire en pelouse calcicole: Une approche historique," in *Dynamique* *et gestion des pelouses calcaires de haute-normandie, Hors collection*, ed. P. Antilogus (Mont-Saint-Aignan: Presses universitaires de Rouen et du Havre), 91–108.

Eriksson, Å., and Eriksson, O. (1997). Seedling recruitment in semi-natural pastures: The effects of disturbance, seed size, phenology and seed bank. *Nord. J. Bot.* 17, 469–482. doi: 10.1111/j.1756-1051.1997.tb00344.x

Grime, J. P. (1988). "The C-S-R model of primary plant strategies — origins, implications and tests," in *Plant evolutionary biology*, eds L. D. Gottlieb and S. K. Jain (Dordrecht: Springer), 371–393. doi: 10.1007/978-94-009-1207-6_14

Heim, A., and Lundholm, J. (2014). The effects of substrate depth heterogeneity on plant species coexistence on an extensive green roof. *Ecol. Eng.* 68, 184–188. doi: 10.1016/j.ecoleng.2014.03.023

Kalamees, R., and Zobel, M. (2002). The role of the seed bank in gap regeneration in a calcareous grassland community. *Ecology* 83, 1017–1025. doi: 10.2307/3071910

Kleyer, M., Bekker, R. M. I, Knevel, C., Bakker, J. P., Thompson, K., Sonnenschein, M., et al. (2008). The LEDA traitbase: A database of life-history traits of the northwest European flora. *J. Ecol.* 96, 1266–1274. doi: 10.1111/j.1365-2745.2008.01430.x

Köhler, M. (2006). Long-term vegetation research on two extensive green roofs in Berlin. *Urban Habitats* 4, 3–26.

Köhler, M., and Poll, P. H. (2010). Long-term performance of selected old berlin greenroofs in comparison to younger extensive greenroofs in Berlin. *Ecol. Eng.* 36, 722–729. doi: 10.1016/j.ecoleng.2009.12.019

Köppen, W. (1923). *Die klimate der erde: Grundriss der klimakunde. Die klimate der erde*. Berlin: Walter de Gruyter & Co. doi: 10.1515/9783111491530

Kowarik, I. (2011). Novel urban ecosystems, biodiversity, and conservation. *Environ. Pollut.* 159, 1974–1983. doi: 10.1016/j.envpol.2011.02.022

Lambinon, J., Delvosalle, L., and Duvigneaud, J. (2012). *Nouvelle flore de la belgique, du grand-duché de luxembourg, du nord de la France et des régions voisines*, 6 Edn. Meise: Editions du Patrimoine du Jardin Botanique National de Belgique.

Lundholm, J., and Richardson, P. J. (2010). MINI-REVIEW: Habitat analogues for reconciliation ecology in urban and industrial environments: Habitat analogues. *J. Appl. Ecol.* 47, 966–975. doi: 10.1111/j.1365-2664.2010.01857.x

Lundholm, J. T. (2006). Green roofs and facades: A habitat template approach. *Urban Habitats* 4, 87–101.

Madre, F., Vergnes, A., Machon, N., and Clergeau, P. (2014). Green roofs as habitats for wild plant species in urban landscapes: First insights from a large-scale sampling. *Landsc. Urban Plan.* 122, 100–107. doi: 10.1016/j.landurbplan.2013.11.012

Milberg, P., and Persson, T. S. (1994). Soil seed bank and species recruitment in road verge grassland vegetation. *Ann. Bot. Fenn.* 31, 155–162.

Monty, A., Jorion, A., Pitz, C., Géron, C., and Mahy, G. (2019). Alien invasive plants in Belgian limestone quarries. *Biotechnol. Agron. Soc. Environ.* 3, 160–164. doi: 10.25518/1780-4507.17984

Oberndorfer, E., Lundholm, J., Bass, B., Coffman, R. R., Doshi, H., Dunnett, N., et al. (2007). Green roofs as urban ecosystems: Ecological structures, functions, and services. *Bioscience* 57, 823–833. doi: 10.1641/B571005

Olly, L., Bates, A. J., Sadler, J. P., and Mackay, R. (2011). An initial experimental assessment of the influence of substrate depth on floral assemblage for extensive green roofs. *Urban For. Urban Green.* 10, 311–316. doi: 10.1016/j.ufug.2011.07.005

Perrier, X., Flori, A., and Bonnot, F. (2003). *Data analysis methods. In genetic diversity of cultivated tropical plants*, trans by P. Hamon, M. Seguin, X. Perrier, and J.C. Glaszmann. Enfield: Science Publishers, 43–76.

Piqueray, J., Bisteau, E., Bottin, G., and Mahy, G. (2007). Plant communities and species richness of the calcareous grasslands in southeast Belgium. *Belgian J. Bot.* 140, 157–173.

R Core Team (2022). *Methodology reference. R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rivière, L., Delruelle, A., Reniers, J., Boisson, S., and Mahy, G. (2022). Disentangling dynamics of green roof vegetation analogue to dry grassland over 3 years: Plant and substrate response to microenvironmental variations. *J. Living Archit.* 9, 1–17. doi: 10.46534/jliv.2022.09.02.001

Roulston, T., Heim, A., and Lundholm, J. (2020). Heterogeneous substrate depth supports greater functional diversity with comparable stormwater retention and substrate temperature services to sedum-dominant green roofs. *J. Living Archit.* 7, 19–39. doi: 10.46534/jliv.2020.07.02.019

Šerá, B., and Šerý, M. (2004). Number and weight of seeds and reproductive strategies of herbaceous plants. *Folia Geobot.* 39, 27–40. doi: 10.1007/BF02803262

Service Fédéral Belge (2019). *Climat.be: Le climat en Belgique*. Available online at: https://www.climat.be/fr-be/changements-climatiques/en-belgique/climat-en-belgique/ (accessed April 17, 2019).

Sutton, R., Harrington, J. A., Skabelund, L., MacDonagh, P., Coffman, R. R., and Koch, G. (2012). Prairie-based green roofs: Literature, templates, and analogs. *J. Green Build.* 7, 143–172. doi: 10.3992/jgb.7.1.143

Sutton, R., and Lambrinos, J. (2015). "Green roof ecosystems: Summary and synthesis," in *Green roof ecosystems ecological studies*, ed. R. K. Sutton (Cham: Springer International Publishing), 423–440. doi: 10.1007/978-3-319-14983-7_17

Tallent-Halsell, N., and Watt, M. S. (2009). The invasive *Buddleja davidii* (butterfly bush). *Bot. Rev.* 75, 292–325. doi: 10.1007/s12229-009-9033-0

Thompson, K., Bakker, J. P., and Bekker, R. M. (1997). *The soil seed banks of North West Europe: Methodology, density and longevity. Vol. 7. Seed science research 3*. Cambridge: Cambridge University Press.

Thuring, C., and Dunnett, N. P. (2019). Persistence, loss and gain: Characterising mature green roof vegetation by functional composition. *Landsc. Urban Plan.* 185, 228–236. doi: 10.1016/j.landurbplan.2018.10.026

Thuring, C., and Grant, G. (2015). The biodiversity of temperate extensive green roofs: A review of research and practice. *Isr. J. Ecol. Evol.* 62, 44–57. doi: 10.1080/15659801.2015.1091190

Vandvik, V., Klanderud, K., Meineri, E., Måren, I. E., and Töpper, J. (2016). Seed banks are biodiversity reservoirs: Species–area relationships above versus below ground. *Oikos* 125, 218–228. doi: 10.1111/oik.02022

Vanstockem, J., Ceusters, C., Van Dyck, K., Somers, B., and Hermy, M. (2018). Is there more than meets the eye? Seed bank analysis of a typical novel ecosystem, the extensive green roof. *Appl. Veg. Sci.* 21, 419–430. doi: 10.1111/avsc.12383

Vanstockem, J., Somers, B., and Hermy, M. (2019). Weeds and gaps on extensive green roofs: Ecological insights and recommendations for design and maintenance. *Urban For. Urban Green.* 46:126484. doi: 10.1016/j.ufug.2019.126484

Willems, J. H., and Bik, L. P. M. (1998). Restoration of high species density in calcareous grassland: The role of seed rain and soil seed bank. *Appl. Veg. Sci.* 1, 91–100. doi: 10.2307/1479088

Williams, N. S. G., Lundholm, J., and Scott MacIvor, J. (2014). FORUM: Do green roofs help urban biodiversity conservation? *J. Appl. Ecol.* 51, 1643–1649. doi: 10.1111/1365-2664.12333

frontiers | Frontiers in Environmental Science

TYPE Original Research
PUBLISHED 22 June 2023
DOI 10.3389/fenvs.2023.1137845

OPEN ACCESS

EDITED BY
Arnaldo Marín,
University of Murcia, Spain

REVIEWED BY
A. M. Keith,
UK Centre for Ecology and Hydrology (UKCEH), United Kingdom
Jana Müllerová,
Jan Evangelista Purkyně University in Ústí nad Labem, Czechia

*CORRESPONDENCE
Quentin Lambert,
qntnlmbrt@gmail.com

RECEIVED 27 January 2023
ACCEPTED 31 May 2023
PUBLISHED 22 June 2023

CITATION
Lambert Q, Bischoff A, Enea M and Gros R (2023), Photovoltaic power stations: an opportunity to promote European semi-natural grasslands?
Front. Environ. Sci. 11:1137845.
doi: 10.3389/fenvs.2023.1137845

Photovoltaic power stations: an opportunity to promote European semi-natural grasslands?

Quentin Lambert[1]*, Armin Bischoff[2], Morgane Enea[1,3] and Raphael Gros[1]

[1]Aix Marseille Université, Université d'Avignon, IRD, CNRS, Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), Campus Etoile, Marseille, France, [2]Université d'Avignon, Aix Marseille Université, IRD, CNRS, Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), IUT Avignon Avignon, France, [3]Université de Sherbrooke, Département de biologie, Centre d'étude de la Forêt, Sherbrooke, QC, Canada

European semi-natural grasslands are particularly species-rich and provide many ecosystem services but depend on extensive land management. Today, these ecosystems are highly threatened by land abandonment, land use intensification and habitat destruction. The increasing construction of solar parks may contribute to habitat destruction but also provide an opportunity to restore or even create semi-natural grasslands. We studied ten solar parks along a climate gradient in Southern France to evaluate the establishment of semi-natural grasslands managed by grazing. We compared plots outside and under solar panels in terms of plant community composition, soil biodiversity and soil functioning to test whether solar panels hamper this establishment. The different microclimate under solar panels strongly affected the plant species composition and reduced the abundance of soil mesofauna and biomass of fungi and gram-negative bacteria. Outside panels, the vegetation was shaped by a climatic gradient, in particular by variations in temperature and precipitation whereas under panels variations were smaller indicating a homogenizing effect of panels on plant community composition. We found more trophic interactions between panels compared to outside and under panels suggesting a protection effect of panels between rows on the soil food web. However, plots under panels showed the lowest number of interactions demonstrating that the strong shading had a negative effect on the plant-soil food web. Solar panels therefore reduce the plant and soil biodiversity of semi-natural grasslands and disrupt ecosystem functions. In order to improve the ecological integration of solar parks, it is thus necessary to mitigate negative effects of solar panels on biodiversity or to increase the space between panels.

KEYWORDS

solar parks, biodiversity, plant, soil, trophic interactions, mesofauna

1 Introduction

Semi-natural grasslands represent 5% of the European and 20% of the French territory (U.E—SOeS, Corine Land Cover 2006). They are among the habitats of the highest species richness for many taxa at community scale (Wilson et al., 2012; Habel et al., 2013) and provide many ecosystem services such as pollination, carbon sequestration, soil conservation and livestock feeding (Bengtsson et al., 2019). The high species richness of herbaceous plants plays a key role in the functioning of this ecosystem and drives its biodiversity by providing resources and ecological niches resulting in a high complexity of interaction networks (Habel et al., 2013; Bengtsson et al., 2019). Semi-natural grasslands are one of the most threatened habitats in Europe (Gang et al., 2014). Land-use intensification (overgrazing, cropland), as well as construction activities (urban sprawl, industrial facilities) or, in contrast, land abandonment resulting in shrub encroachment, have led to a dramatic loss of European semi-natural grasslands. The rapid development of renewable energy in Europe such as solar park construction may further increase the pressure on semi-natural grasslands. Van de Ven et al. (2021) estimated that 3% of open habitats such as grasslands need to be transformed to solar parks in order to reach the European Union objectives for solar energy production. However, there may also be a chance for promoting semi-natural grasslands if solar parks are constructed in degraded habitats such as monospecific forests, quarries, arable land or wasteland providing an opportunity to benefit biodiversity and ecosystem functioning of new semi-natural grasslands (Hernandez et al., 2019; Lambert et al., 2022).

Semi-natural grassland biodiversity and functioning are driven by climate, soil and land use (Zarzycki and Bedla, 2017; Basile-Doelsch et al., 2020; Boonman et al., 2021). Climatic and abiotic soil conditions are also important drivers of biotic soil functioning and plant-soil interactions (Craine et al., 2012). Plant diversity affects decomposer organisms by resource diversification (beetles, mites, springtails, microorganisms) (Zak et al., 2003; Isbell et al., 2017). Steinauer et al. (2015) showed that higher plant diversity is closely related to higher microbial activity and biomass. The plant functional diversity also drives the diversity of the mesofauna decomposers (Moradi et al., 2017). These decomposers contribute to the structure of grassland plant communities by feeding on roots and translocating nutrients (Whiles and Charlton, 2006). Moreover, the mesofauna decomposer abundance directly affects mesofauna predators (such as mites, diplurans). Soil mesofauna such as plants and microorganisms are also influenced by climate (Barnett and Facey, 2016). The high floristic and faunistic biodiversity of semi-natural grasslands depends on extensive grazing or mowing avoiding shrub encroachment and a succession towards woody plant communities (Pulungan et al., 2019). The abandonment of such management leads to a strong decrease of plant diversity and related soil functions (Malcolm et al., 2006).

The construction of solar parks and subsequent use for electricity production changes environmental conditions (Hernandez et al., 2014). Clearing and vegetation management by grazing hampers shrub encroachment resulting in a dominance of herbaceous plant species (Armstrong et al., 2016). The construction of solar parks further leads to a degradation in the soil physico-chemical quality involving a reduction of aggregate stability and a compaction of the topsoil layer (Lambert et al., 2021). Choi et al. (2020) showed that total carbon and nitrogen content were two times lower in soils of solar parks than in arid grasslands. Solar panels also change microclimate resulting in a reduction of light, soil temperature and moisture (Uldrijan et al., 2021; Lambert et al., 2022; Moscatelli et al., 2022). Plant biomass and diversity were lower under solar panels while mortality and chlorophyll content were higher (Uldrijan et al., 2021; Lambert et al., 2022; Moscatelli et al., 2022). The lack of light under solar panels favors shade-tolerant species (Lambert et al., 2022) resulting in semi-natural vegetation types that can be found close to forests or in pasture woodlands. Whereas solar radiation is always reduced under solar panels, temperature and soil moisture may also be higher, for example, during the night (temperature) or after drought periods (soil moisture) thus buffering extreme values (Lambert et al., 2021). This buffering effect on microclimate may change the relationship between vegetation and regional climate.

The lower nutrient content in solar parks and the changes in microclimate under panels also change soil microbial biomass and enzyme activities involved in N and C cycling (Lambert et al., 2021; Moscatelli et al., 2022). The effect of solar panels on vegetation and soil microorganisms leads to a reduction in soil CO_2 emission (Lambert et al., 2021) affecting biomass production (Armstrong et al., 2016). However, a better understanding of the decomposer food web and its interactions with plants is needed to evaluate consequences of solar park construction and solar panels on ecological functioning of semi-natural grasslands such as carbon cycling and sequestration.

In order to evaluate limits and chances of solar park construction and exploitation for the establishment of semi-natural grasslands, we compared plots outside and under solar panels in terms of plant community composition, soil organisms and soil functioning along a climate gradient. We expected that 1) the plant community composition outside solar panels is driven by climate and that the climate effect on plant communities is smaller under panels, 2) solar panels strongly affect plant communities and soil parameters. We finally hypothesized that 3) the changes in plant and soil communities disintegrate the soil food web network.

2 Materials and methods

2.1 Study sites and sampling design

This study was set up in ten solar parks (SPs) located in Southern France along the Rhône valley (Figure 1). The SPs are distributed along a climate gradient from the Mediterranean Sea in the south to inland sites in the north and east (Table 1). Distance to the sea was between 37 and 192 km, and sites differed in major climate characteristics from Mediterranean in the south to sub-Mediterranean at highest distances from the coastline (Table 1). Continentality increased to the north, mean annual temperature ranged from 11.8°C to 14.9°C and annual precipitation from 710 mm to 903 mm. Other environmental factors such as soil and technical characteristics are similar between SPs. The size of the SPs ranged from 1 to 7 ha, equipped with solar panel either fixed on the ground or trackers. Ground-fixed panels had a height of 1 m at the southern edge and of 2.5 m at the northern edge (inclination towards south) whereas the incliniation of tracker panels changed according to the sun position. The SP were built on former wasteland, crop land or semi-natural land (Table 1). The solar panels were aligned to form rows with a gap of 2.5 m between rows. All solar parks were managed by extensive sheep grazing. Parks were additionally mown when grazing was not intensive enough or irregular.

Within each SP, five blocks of at least 50 m distance were set up in spring 2021. In each block (Supplementary Appendix S1), three

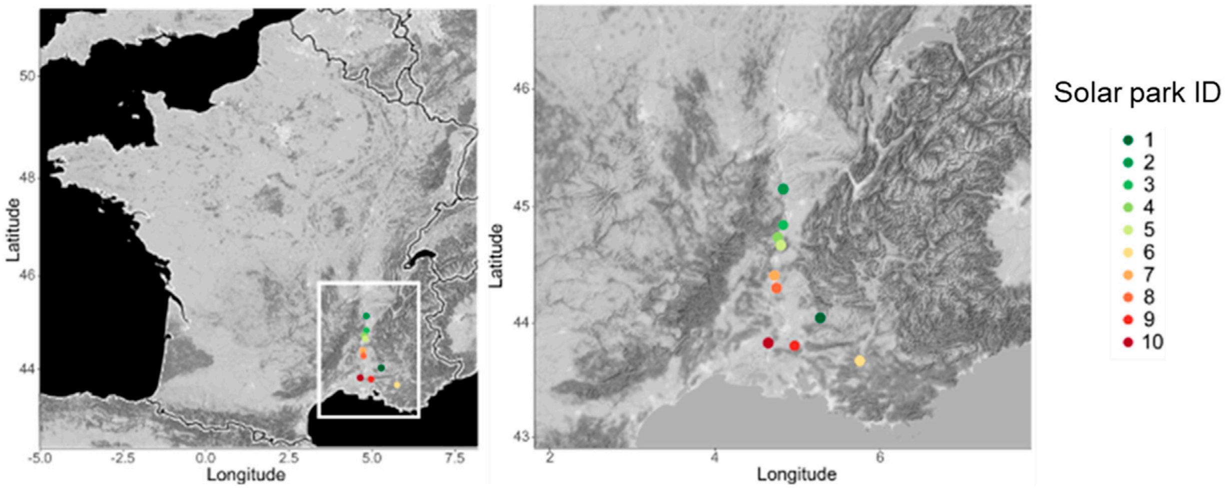


FIGURE 1
Geographical position of solar parks. Colors of SP position represent a mean annual temperature gradient.

TABLE 1 Environmental characteristics of solar parks.

ID	Sites	Solar radiation (kWh/m²/y)	Annual mean temperature (°C)	Annual precipitation (mm)	Distance to the sea (km)	Altitude (m a.s.l.)	Soil type (WRB)	Past land use (CLC nomenclature)
1	Blauvac	1894.19	11.82	863	80	570	Calcisol	Sclerophyllous vegetation (323)
2	Ozon	1411.33	12.43	832	192	130	Fluvisol	Natural grasslands and pastures (321)
3	Saint-Georges-les-Bains	1522.73	12.74	876	158	107	Fluvisol	Complex cropping systems and parcels (242)
4	Le Pouzin	1540.99	12.97	895	145	91	Fluvisol	Complex cropping systems and parcels (242)
5	Les Tourettes	1578.98	12.93	903	138	85	Fluvisol	Complex cropping systems and parcels (242)
6	Saint-Paul-Lez-Durance	1877.75	13.07	710	57	388	Rendosol	Changing forests and shrubby vegetation (324)
7	Donzères	1718.5	13.64	870	108	68	Calcosol	Natural grasslands and pastures (321)
8	Bollene	1760.87	13.99	843	96	64	Fluvisol	Changing forests and shrubby vegetation (324)
9	Mollégès	1843.78	14.42	720	46	58	Calcisol	Complex cropping systems and parcels (242)
10	Tarascon	1775.85	14.94	743	38	18	Fluvisol	Grassland (231)

plots of 4 m^2 were delimited for the following treatments: outside solar panels (outside), between two rows of solar panels (between) and under solar panels (under) (N = 10 solar parks X 5 blocks X 3 treatments = 150).

2.2 Vegetation monitoring

The effect of solar panels on plant community composition and vegetation cover (vertical projection of above-ground plant organs) of all vascular plant species was evaluated in each plot in late spring. To evaluate the effects of solar panels on physiological performance of the vegetation, a chlorophyll index was measured by Multiplex® 3 (FORCE-A, Orsay, France) with ten records of chlorphyll fluorescence within each plot. The Multiplex ResearchTM allows *in situ*, non-destructive and real-time measurements of chlorophyll content (Agati et al., 2011).

2.3 Soil sampling and physico-chemical properties

Soil was sampled in the same plots as the vegetation. In April 2021, three soil samples were randomly collected (10 cm depth) in each plot and mixed to one bulk sample. Bulk samples were sieved (mesh size: 2 mm) prior to analysis. An aliquot of samples was air-dried (1 week, 30 C) for physico-chemical analyses and another aliquot stored at 4°C for microbial analyses. Soil water content was determined after drying samples (24 h, 105°C). Total carbon (TC) and total nitrogen (TN) contents were measured by combustion in the CN FlashEA 1112 (ThermoFisher) (NF ISO 10694, NFISO 13878) elemental analyser.

2.4 Sampling and determination of springtails and mites

In April 2021, the soil mesofauna was sampled in stainless steel tubes (5.5 cm diameter, 6 cm depth) using two core samples per plot after vegetation removal. Mites and springtails were extracted during a period of 7 days at 25°C using the Berlese-Tullgren method (Bano and Roy, 2016) and then stored in 70% ethanol. Springtails and mites were counted under a stereomicroscope.

2.5 Microbial community composition and enzymatic activities

Phospholipid fatty acids (PLFA) are essential components of all living cells, but several PLFA are specific to bacterial and fungal biomass. PLFA were analyzed according to Buyer and Sasser (2012) and Aupic-Samain et al. (2021). After freeze-drying, 1 g of dry soil of each sample was extracted in 4 mL of Bligh–Dyer extractant containing 4 µL of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (C19:0; Avanti® Polar lipids, Inc.). Lipids were separated using solid-phase extraction (SPE) on Phenomenex® (Strata SI-1 with 50 mg of silica, 55 µm, 70 Å). The resulting fatty acids methyl ester (FAME) were analysed by gas-chromatography/mass-spectrometry (GC-MS) on an Agilent 7890 system equipped with an MSD5977. A network mass detector, an ALS7693 automatic injector and an HP5-MS apolar column (30 m × 0.25 mm × 0.25 µm; JandW Agilent Technologies). Qualitative analysis of FAMEs resulted from retention time comparison of the FAME mixture (range between C4 to C24). We analyzed 85 specific biomarkers out of 98 identified PLFAs. Each biomarker was attributed to a specific taxon such as arbuscular mycorrhiza, other fungi, actinobacteria, gram-negative bacteria, gram-positive bacteria, in accordance with Frostegård et al. (1993). Bacterial biomass was calculated as the sum of actynobacteria, gram-negative and gram-positive bacteria biomass. Total microbial biomass was calculated as the sum of fungal and bacterial biomass. The details of the attribution of each peak to the taxa are presented in Supplementary Appendix S2.

The activity of luorescein diacetate hydrolase (FDAse) was measured according to Green et al. (2006) in order to determine the catabolic potential of microbial communities. FDAse is a complex of enzymes comprising phosphatase, cellulase, and lipase involved in the decomposition of cellulose and carbohydrates (Guénon et al., 2017).

2.6 *In situ* soil respiration

In May 2021, *in situ* soil CO_2 effluxes ($g.CO_2.m^{-1}.h^{-1}$) were recorded using a portable gas analyser (EGM-4, PP-system) after removal of aboveground vegetation. The soil CO_2 effluxes represent the respiration of plant roots, soil organisms and chemical oxidation of C compounds. The device was connected to a closed soil respiration chamber (SRC-1, PP systems Massachusetts, United States). To prevent leakage of CO_2 when placing the chamber on the soil, a PVC tube (10 cm × 11 cm) was buried 1 cm deep into the soil prior to measurements. In each plot, soil temperature was recorded *in situ* in a depth of 7 cm using the soil temperature probe (STP-1, PP-system) connected to the portable gas analyzer.

2.7 Statistical analyses

Climatic variables were extracted from WorldClim version 2.1 dataset using the period from 1970 to 2000 (Fick and Hijmans, 2017). The 19 climatic variables were extracted on a grid of 1 km^2 and represented annual means of temperature and precipitation, seasonality, and extreme values resulting from MODIS images of the same period.

All data were analysed using R software (3.6.1, R core Team, 2020). Relationships between plant community composition, bioclimatic variables, plot position (under vs. outside panels) and one-way interaction of plot position and climatic variables were analysed using Redundancy analysis (RDA) in the R package "vegan" (Oksanen et al., 2007). Prior to RDA, multicollinearity of the 19 WorldClim explanatory variables and of their interactions with panel was tested by calculating the variance inflation factor (VIF) and using a cut value of 3. VIF retained four of them as non-collinear (Precipitation of Wettest Month, Annual Temperature Range, Mean Temperature of Wettest Quarter, Temperature Seasonality). A first RDA model tested the effect of panel and these four climatic variables on plant community composition including panel-climate interactions. Starting with an initial model of explanatory variables and interactions not showing collinearity, a backward selection procedure was applied using the "adespatial" package. Significance of explanatory variables was tested using PERMANOVA. Since most climatic variables showed significant interactions with plot position, separate RDA were additionally run for plots under and outside panels to explore the influence of climatic gradients.

To analyze the effect of solar panels on plant community characteristics, Shannon index, Simpson index and evenness were

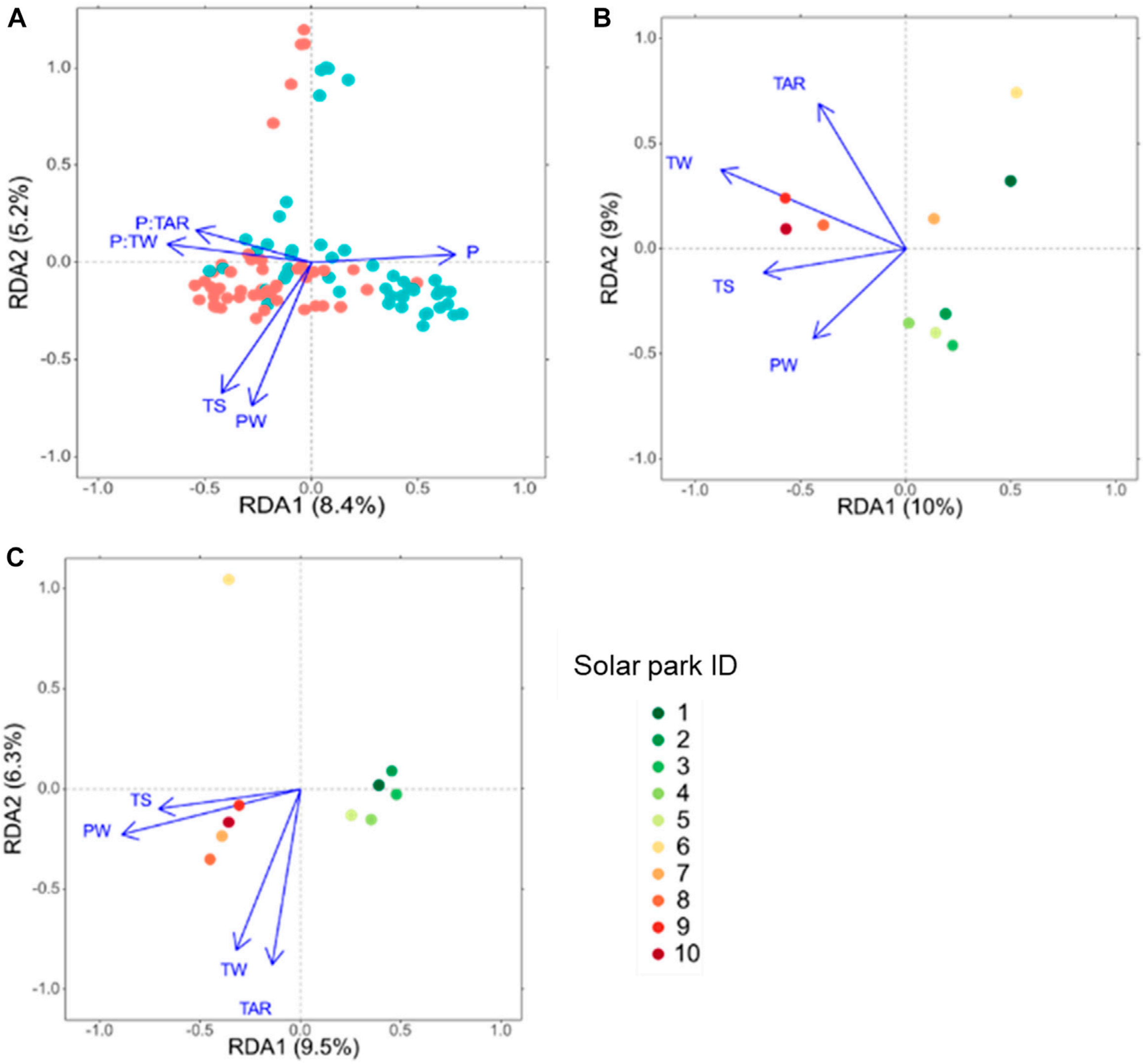


FIGURE 2
Redundancy analyses (RDA) of environmental variables on plant communities in solar parks including panel (P) effect **(A)** and separately outside **(B)** and under solar panels **(C)**. Climate variables were precipitation of the wettest months (PW), temperature seasonality (TS) and the interaction of solar panels with annual temperature range (P:TAR) and temperature of the wettest quarter (P:TW). Red and blue points in panel A are plots under and outside solar panels, respectively. In figure B and C, colored points represent centroids of solar park vegetation.

calculated. For each species, ecological indicator values were obtained using Julve (1998) adapted from Ellenberg et al. (1992). We separated species according to light indicator values in two groups: shade tolerant (1000–5,000 lux) and heliophilous species (>5,000 lux). We tested the habitat preference of each species in order to evaluate whether solar panels change grassland communities to shade-tolerant understory vegetation. The following response variables were tested: diversity indices, total vegetation cover, cover of shade-tolerant and heliophilous plants, chlorophyll index, mite and springtail abundance, biomass of microbes (AM fungi, Total Fungi, total Bacteria, Actynobacteria, Gram –, Gram +), FDase activity, soil respiration, soil temperature and moisture, total carbon and total nitrogen N. Linear mixed-effect models (LMMs) were calculated including position (under vs. between vs. outside panels) as fixed factor and SP as random factor. A Tukey post-hoc test was run to test for differences between the three positions when a significant effect of solar panel was detected. When necessary, data were transformed using the “bestNormalize” package (Peterson, 2021) to meet the assumptions of normality and homoscedasticity of variances.

To evaluate the influence of vegetation cover and main climatic variables on soil food web and soil CO_2 effluxes (a key soil function), a path analysis was performed using R package “lavaan” (Rosseel, 2012). The analysis was run separately for measurements outside, between and under solar panels. Path analysis is a structural equation modelling (SEM) technique used to show causal relationships between several measured variables. To build a full model (Supplementary Appendix S3), we assumed a causal relationship between climatic variables (i.e., annual mean temperature and precipitation) and vegetation cover on total carbon and total nitrogen content, total bacterial and fungal biomass, abundance of springtails and mites, and soil respiration. The full models were simplified by progressively excluding insignificant variables until an appropriate minimum model was obtained. The fit of each model was evaluated by the significance of differences between the predicted and observed covariance matrices (χ^2-squared tests, $p > 0.05$), by the mean square root error of the approximation index (RMSEA <0.1), by comparative fit index (CFI >0.90) and the Tucker–Lewis index (TLI >0.90) (Xia and Yang, 2019).

3 Results

3.1 Solar panel effects on plant community composition along a climatic gradient

Solar panels significantly affected plant community composition and the interactions of panels with annual temperature range (P:TAR) and temperature of the wettest month (P:TW) (Table 3; Figure 2A). Solar panels and interactions constrained the first axis of the RDA. The second RDA axis was constrained by temperature seasonality (TS) and precipitation of the wettest month (PW). Plant communities under solar panels showed less variation on the first RDA axis than those outside solar panels. Vegetation under solar panels was characterized by *Anisantha sterilis* and outside solar panels by *Lotus dorycnium*.

Outside solar panels (Figure 2B), temperature of the warmest month (TW), temperature seasonality (TS) and precipitation of the wettest month constrained the first axis of the RDA, while annual temperature range (TAR) constrained the second one (Figure 2B). The solar parks 1 and 6 were negatively correlated to precipitation of the wettest month and temperature seasonality. And they were characterized by *L. dorycnium*. Solar parks 2 to 5 were positively correlated to PW and negatively to TAR. Solar parks 8 to 10 were positively correlated to TW and TS and were characterized by *Cynodon dactylon* and *Bromus rubens*.

Under solar panels, TS and PW constrained the first axis, while TAR and TW constrained the second axis (Figure 2C). Solar panels 1–5 characterized by *A. sterilis*, grouped together at low precipitation of the wettest month and low temperature seasonality. The solar parks 7 to 10 characterized by *L. dorycnium* also grouped together but at the left part of the biplot characterized by higher precipitation of the wettest month and higher temperature seasonality. The solar park 6 was apart from the others occurring in the upper part of the biplot that was related to lower temperatures of the warmest month and characterized by *Erigeron sumatrensis/canadensis* and *Argyrolobium zanonii*.

Plant communities of solar parks were dominated by ruderal and grassland species. Herbaceous species cover outside and under solar panels was 70 and 10 times higher, respectively, than forest type species cover (Table 2; Supplementary Appendix S5). Two non-native species (*E. sumatrensis/canadensis, Senecio inaequidens*) were recorded in 55 plots. Their cover represented on average 2% of total vegetation cover (Table 2; Supplementary Appendix S5).

The species richness (Figure 3A) and total vegetation cover (Figure 3B) were significantly lower under solar panels than outside panels ($p < 0.05$). The chlorophyll index (Figure 3C; Supplementary Appendix 6) increased under solar panels. The cover of shade-tolerant species (Figure 3D) was twice as high and the cover of heliophilous species (Figure 3E) 2.5 times lower ($p < 0.001$) under solar panels than outside. The cover of non-native species was four times higher ($p < 0.001$) under than outside and between solar panels (Figure 3F). Shannon index, Simpson index and evenness were significantly higher outside than under solar panels (Supplementary Appendix S6).

3.2 Effects of solar panels on soil physico-chemical and biological properties

Total mite abundance was higher ($p < 0.001$) outside than under or between solar panels (0.5 and 1.5 times, respectively, Figure 4A; Supplementary Appendix S6). Springtails were 1.5 times more abundant ($p < 0.001$) outside than under solar panels (Figure 4B; Supplementary Appendix S6). Soil respiration was 5.5 times lower under than between and outside solar panels (Figure 4L, $p < 0.001$). Fungi and gram-negative bacteria biomass were significantly reduced by 25% under solar panels compared with outside (Figures 4C, G), Supplementary Appendix S6). Total biomass of microorganisms was 1.25 times higher outside than under solar panels (Supplementary Appendix S6; Figure 4J). FDAse activity was 20% lower under than between and outside solar panels (Supplementary Appendix S6; Figure 4K).

Solar panels further decreased soil temperature, total carbon and total nitrogen contents but did not significantly change soil water content and C:N (ratio (Table 3; Supplementary Appendix S6). Between solar panels, the soil temperature was about 30% higher than under solar panels Table 4.

No significant differences were found between the different modalities for AM fungi, Bacterial biomass, Actinobacteria, Gram

TABLE 2 Effect of solar panels on plant community characteristics such as habitat preference, plant type and species origin. Mean values with standard errors in parentheses. Different letters indicate significant differences between habitat, plant type and position outside, between and under panels.

	Habitat			Plant type			Species origin	
	Ruderal habitats	Grassland	Forest	Herbaceaous	Shrub	Tree	Native	Alien
Outside	38.26 (±4.17)^a	31.18 (±2.72)^b	1.43 (±0.58)^c	70.66 (±4.64)^a	1.47 (±0.81)^b	0.20 (±0.11)^c	72.05 (±4.74)^a	0.57 (±0.31)^b
Between	32.85 (±3.51)^a	31.43 (±2.8)^a	0.37 (±0.16)^b	64.59 (±4.28)^a	1.34 (±0.59)^b	0.16 (±0.11)^c	65.60 (±4.28)^a	1.01 (±0.33)^b
Under	27.30 (±2.88)^a	28.36 (±3.57)^a	1.39 (±0.54)^b	56.79 (±4.04)^a	5.08 (±1.57)^b	0.31 (±0.21)^c	60.19 (±4.26)^b	3.96 (±1.00)^a

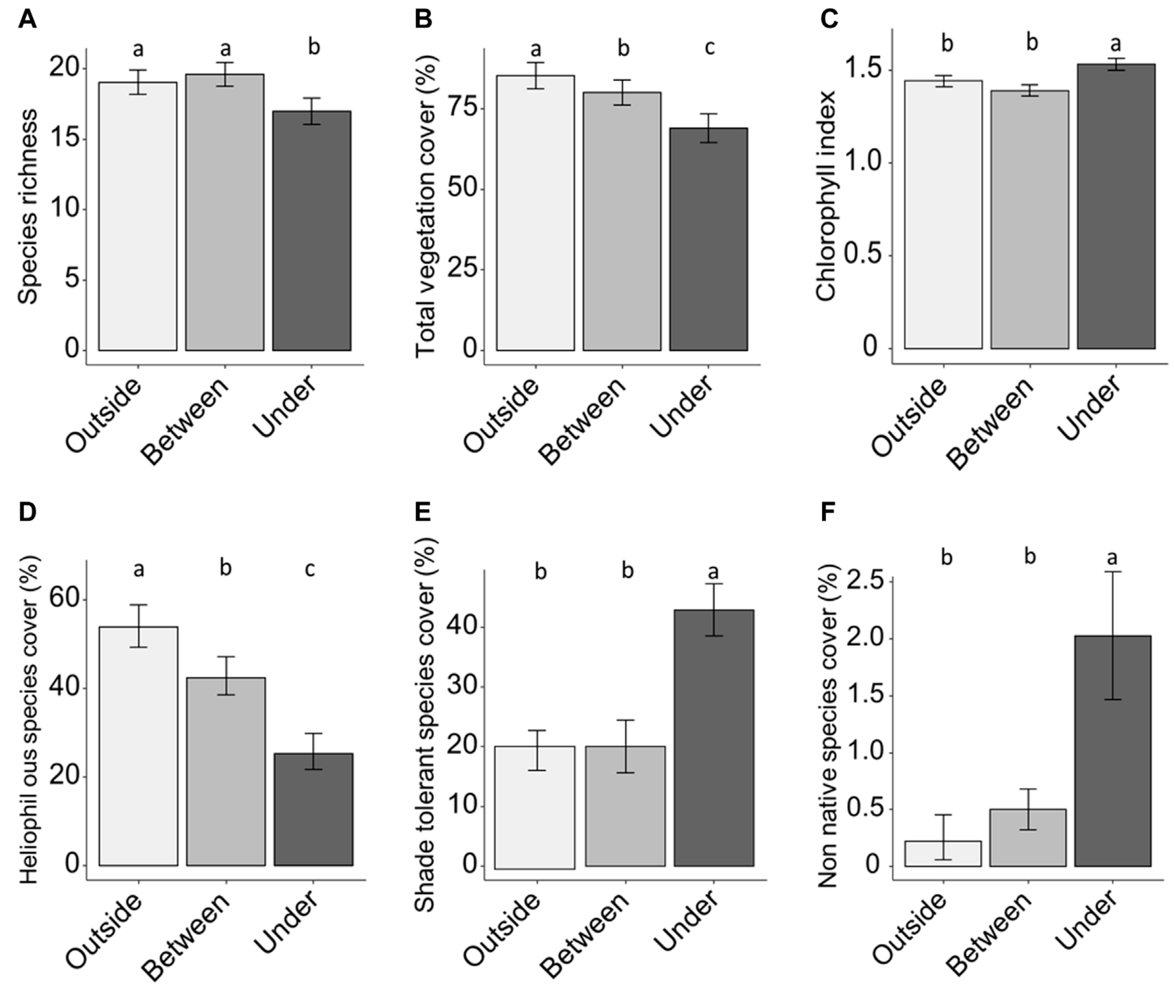


FIGURE 3
Species richness **(A)**, total vegetation cover **(B)**, chlorophyll index **(C)**, total cover of shade-tolerant species **(D)**, total cover of heliophilous species **(E)**, total cover of non-native species **(F)** outside, between and under solar panels. Error bars are means ± SE; different lower-case letters indicate significant differences ($p < 0,05$).

positives biomass, Fungi:Bacteria and Microbial biomass (respectively Figures 4D–F, H, I).

3.3 Effects of solar panels on interaction networks

Outside panels, the most parsimonious model for predicting soil bacterial biomass, springtail abundance and CO_2 effluxes included precipitation, temperature, plant cover and total C contents as significant explanatory variables. The model was characterized by ten significant relationships. Plant cover, the abundance of soil organisms and CO_2 effluxes were mainly controlled by mean annual temperature and precipitation. Annual precipitation had an indirect effect on CO_2 effluxes via the influence on bacterial biomass (−), vegetation cover (+), and soil organic carbon concentrations (+) (Figure 5A). An increase in vegetation cover and then total C content led to an increase in springtail abundance.

Between solar panels, the most parsimonious model for predicting soil bacterial biomass, abundance of mesofauna organisms and CO_2 effluxes included precipitation, plant cover and C and N contents as significant explicative variables (Figure 5B). The model is characterized by ten significant relationships. Plant cover, abundance of soil organisms and CO_2 effluxes were mainly controlled by mean annual precipitation. Collembola and mites were affected by organic carbon (+) and nitrogen content (−) and springtails additionally by vegetation cover (+). CO_2 effluxes increased with fungi biomass increase and bacterial biomass decrease.

Under panels, the most parsimonious model for predicting soil bacterial biomass and abundance of mesofauna organisms included precipitation, temperature, plant cover and C and N contents as significant explicative variables (Figure 5C). The model is characterized by seven significant relations. Annual mean temperature and precipitation controlled microbial biomass (−) only. Vegetation cover directly controlled (−)

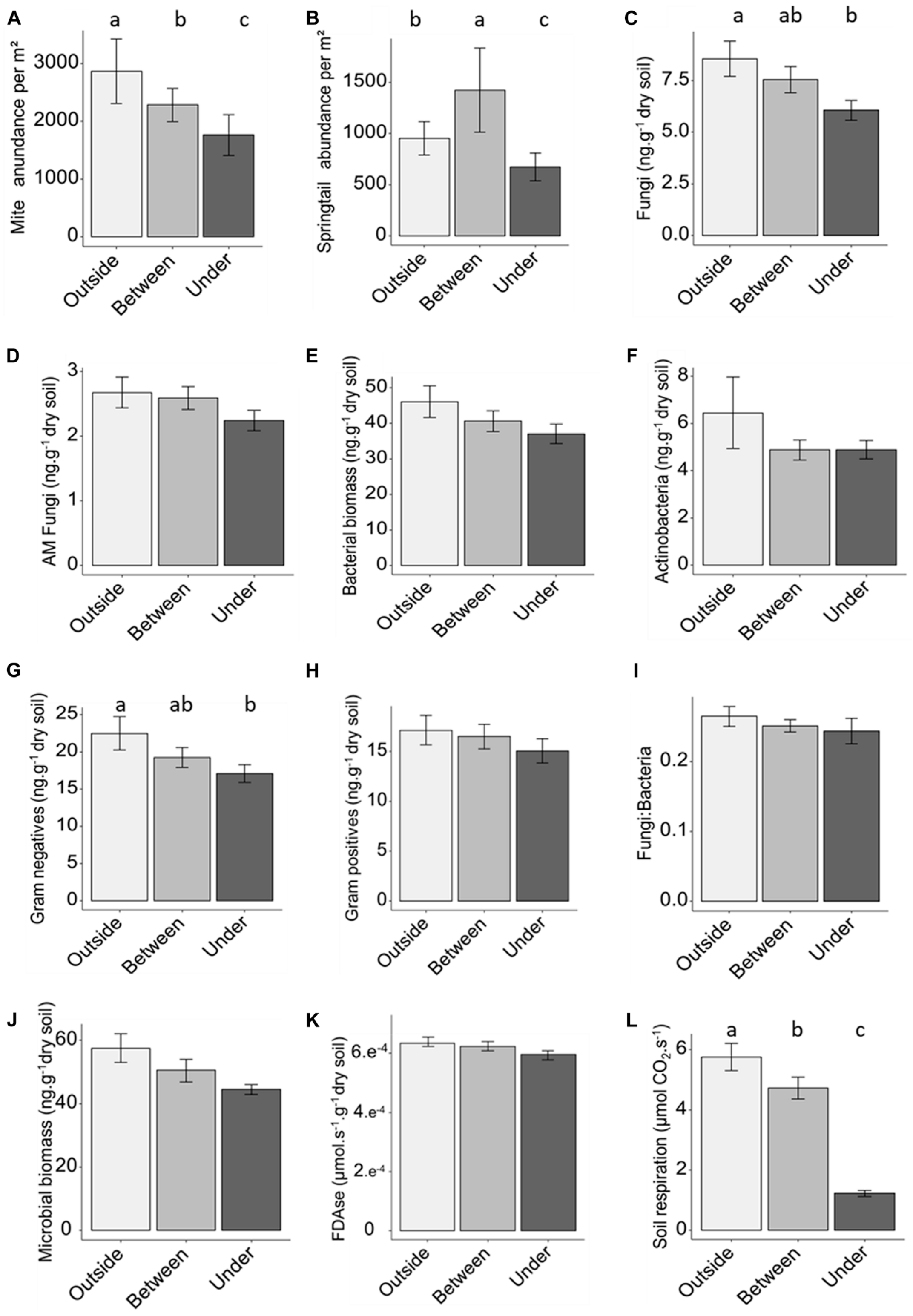


FIGURE 4
Mite abundance **(A)**, springtail abundance **(B)**, total fungi biomass **(C)**, arbuscular mycorrhiza biomass **(D)** total bacteria biomass **(E)**, actinobacteria biomass **(F)**, gram-negative bacteria biomass **(G)**, gram-positive bacteria biomass **(H)**, fungi:bacteria ratio **(I)**, total microbial biomass **(J)**, fluorescein diacetate hydrolase activity **(K)**, soil respiration **(L)** outside, between and under solar panels. Error bars are means ± SE; different lower-case letters indicate significant differences ($p < 0.05$).

TABLE 3 PERMANOVA results (RDA) of panel and climate effects on plant communities of solar parks. Degree of freedom, ANOVA F values and significance: . $p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. P: solar panels, PW: precipitation of the wettest month, TAR: annual temperature range, TS: temperature seasonality, TW: temperature of the wettest quarter.

	Properties	Df	F	*p*-value
RDA constrained by solar panel	Global RDA	5	5.20	***
	RDA 1	1	9.83	***
	RDA 2	1	6.10	***
	P	1	6.35	***
	PW	1	5.69	***
	TS	1	5.75	***
	P:TAR	1	2.11	***
	P:TW	1	2.38	***
Outside solar panels RDA	Global RDA	3	4.41	***
	RDA 1	1	6.05	***
	RDA 2	1	5.44	***
	PW	1	3.49	***
	TAR	1	4.64	***
	TS	1	4.85	***
	TW	1	4.66	***
Under solar panels RDA	Global RDA	4	3.26	***
	RDA 1	1	5.44	***
	RDA 2	1	3.57	***
	PW	1	2.99	***
	TAR	1	3.43	***
	TS	1	3.88	***
	TW	1	2.75	**

mite abundance and indirectly (+) springtail abundance by an increase in organic carbon. Although bacterial biomass decreased with increasing precipitation, CO_2 effluxes under panels were explained by climatic, chemical, and biological variables. Similarly to the SEM outside and between panel, no significant interaction between bacterial biomass and mesofauna abundance was detected.

4 Discussion

Plant community composition outside solar panels was driven by climatic variables and reflected the climate gradient. Vegetation under solar panels varied less between the solar parks than vegetation outside panels indicating a homogenizing effect of panels on plant community composition. Solar panels affected the soil mesofauna and two microbial groups (fungi and gram-negative bacteria), and reduced the effects of annual precipitation and temperature on soil CO_2 effluxes.

4.1 Changes in plant community composition along a climatic gradient in solar parks

In the studied solar parks, plant communities outside panels were distributed along a climatic gradient. Through grading and clearing of vegetation, both soil quality and plant community composition change during the construction of solar park (Armstrong et al., 2016; Lambert et al., 2021). Plant establishment and spatial distribution of communities after a disturbance are driven by local climatic and soil physico-chemical parameters (Zarzycki and Bedla, 2017; Boonman et al., 2021). Chauvier et al. (2021) showed that artificial land cover (*i.e.*, urban and agricultural area) clearly affects plant distribution along a climatic gradient in lower parts of the European Alps. Our results showed that climatic variables such as precipitation of the wettest month and temperature annual range still drive plant community composition of solar parks suggesting that solar parks do not totally disintegrate the relationship between climate and vegetation.

Outside the panels, vegetation was shaped by a climatic gradient, in particular by variations in temperature and precipitation. More precisely, we found that plant community composition was driven by the mean temperature of wettest quarter, temperature seasonality, annual temperature range and the precipitation of the wettest month. Our results suggest a progressive decrease in the sensitivity of plant species towards lower latitude to an increase in temperature variability and in the range of extreme temperature and precipitation conditions characteristic for the more continental climate of our inland sites (Maestre et al., 2009). The solar panels affected plant community composition and formed two distinct communities shaped by temperature. Under panels, mean temperature of the wettest month and annual temperature range had a smaller effect on plant communities than outside panels. Plant composition of grasslands is often driven by climate-related water

TABLE 4 Effect of solar panels on physico-chemical characteristics. Mean values with standard errors in parentheses. Different letters indicate significant differences ($p < 0.05$) between positions outside, between and under panels.

	Outside solar panels	Between solar panels	Under solar panels
Soil Temperature (°C)	19.18 (±0.45)^a	18.02 (±0.45)^b	14.50 (±0.21)^c
Soil water content (%)	9.03 (±1.10)	10.20 (±1.04)	9.83 (±0.88)
Total Carbon content (%)	6.09 (±0.37)^a	5.45 (±0.38)^{ab}	5.09 (±0.32)^b
Total Nitrogen content (%)	0.28 (±0.03)^a	0.24 (±0.02)^{ab}	0.23 (±0.02)^b
C:N	28.64 (±2.79)	26.95 (±1.78)	25.10 (±2.73)

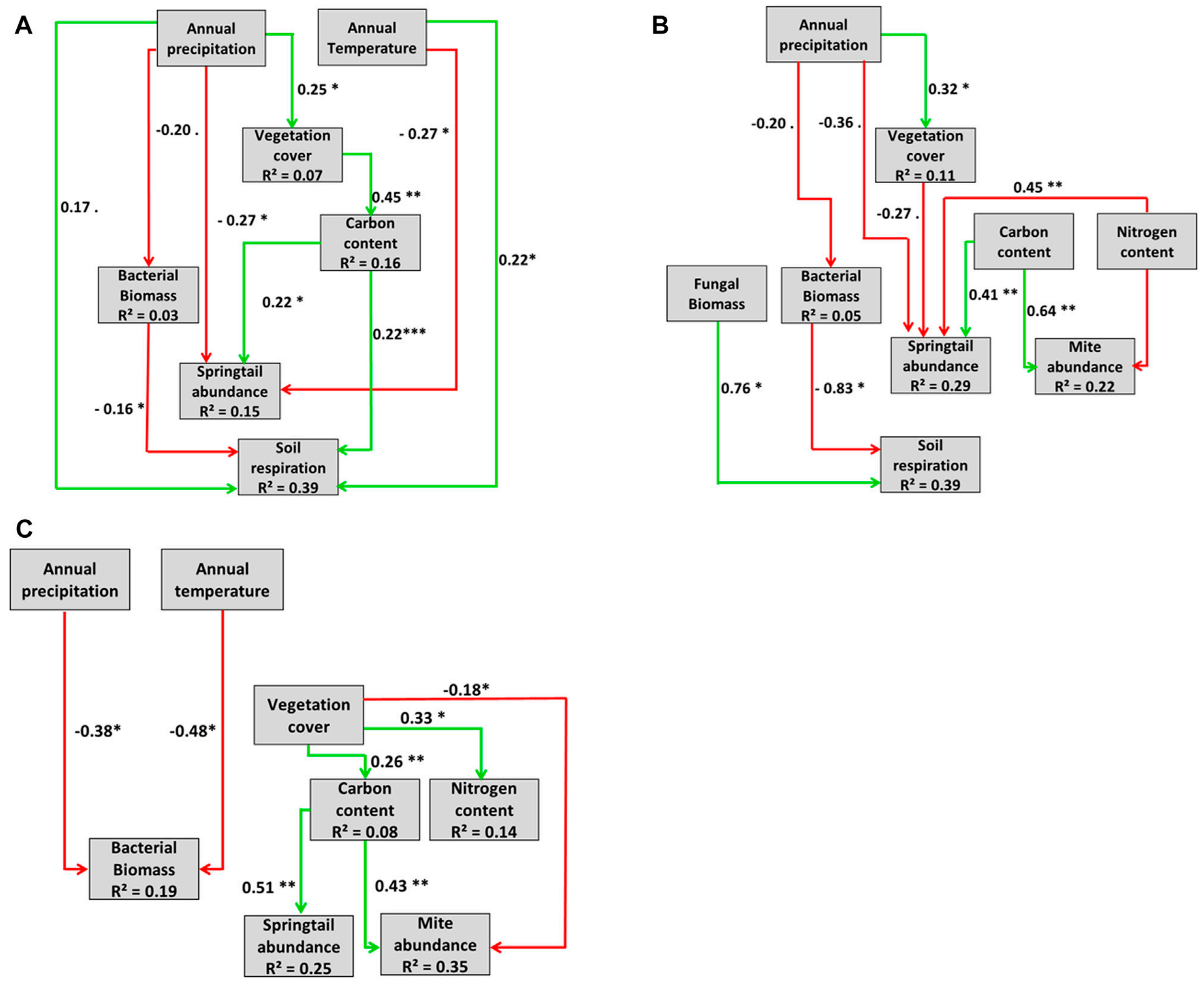


FIGURE 5
Path analysis models. Causal influence of mean annual precipitation (mm) and temperature (°C) on vegetation cover (%), total nitrogen content (N%), total carbon (C%), bacterial biomass, fungi biomass, springtails abundance, mites abundance and soil respiration (μmolCO$_2$. s-1) **(A)** outside (d.f. = 9, χ^2 = 3.26, p = 095, RMSEA = 0.00, CFI = 1.00, TLI = 1.42), **(B)** between solar panels (d.f. = 21, χ^2 = 31.17, p = 0.07, RMSEA = 0.09, CFI = 0.94, TLI = 0.90), and **(C)** under solar panels (d.f. = 11, χ^2 = 12.02, p = 0.36, RMSEA = 0.04, CFI = 0.99, TLI = 0.97). Green arrows represent positive, red arrows negative relationships. Significance levels are indicated as ***$p < 0.001$, **$p < 0.01$, $p < 0.05$, .$p < 0.1$). Non-significant paths ($p > 0.1$) were eliminated.

availability (Cherwin and Knapp, 2012; Cleland et al., 2013). Armstrong et al. (2016) reported a buffer effect of panels on climatic variations leading to a reduction of ecological niche amplitude and a decrease in plant richness under a temperate-oceanic climate. We also observed a decrease in plant species richness under solar panels. In semi-natural dry grasslands, plants are often more affected by the lack of precipitation during the wettest months in autumn and winter (Dostalek and Frantik, 2011; Fischer et al., 2014). Rainfall during these periods is essential for seed germination and provides a soil water stock for the dry summer. Reduced rainfall under the panels may have limited plant establishment (Akinci, 2013; Lambert et al., 2022).

Contrary to Uldrijan et al. (2022) who showed that the cover of heliophilous plants was greater under the panels in the Czech Republic, we found that the light reduction under panels resulted in a shift from heliophilous to shade-tolerant plant species that are more competitive under such conditions (Chen et al., 2004). This reduction of light also resulted in an increase in chlorophyll content, confirming the results of Lambert et al. (2022) who found a higher chlorophyll content in leaves of *Brachypodium retusum* under panels. Solar panels change the metabolism of the plants that need to allocate more resources to the production of chlorophyll in order to maintain a sufficient photosynthetic activity under panels (Ma et al., 2010). This higher resource allocation in chlorophyll production may have resulted in a reduction of leave, root and flower biomass. Total vegetation cover and species richness were smaller under solar panels which may be a consequence such a reduction in plant performance. These changes in plant performance and plant community composition may affect ecological functioning. Uldrijan et al. (2022) showed that plant species under solar panels had fewer interactions with other taxa than species than outside panels. Such changes in the number of interactions may negatively affect ecosystem services provided by natural grasslands such as carbon sequestration (Uldrijan et al., 2022).

4.2 Effects of solar panels on soil physico-chemical and biological properties

During daytime, we measured a clear reduction of temperature by 4°C under solar panels confirming results of Moscatelli et al. (2022) and Lambert et al. (2021) under similar Mediterranean climate. However, continuous measurements showed a homogenizing effect of solar panels on temperature being lower during daytime but higher at night (Armstrong et al., 2016). Solar panels intercept rainfalls but reduce evapotranspiration (Lambert et al., 2021) resulting in non-significant panel effects on soil moisture. Accordingly, the area between panels (inter-rows) still affected by shading of panels but not protected from rainfall showed the highest soil moisture. Effects of panels on soil temperature and moisture may further depend on seasonal variations (Armstrong et al., 2016). Lower C and N contents were observed under panels resulting from a lower plant biomass production and subsequently a lower organic matter content.

We found that FDAse activity, soil respiration and microbial biomass were reduced under solar panels. Climate and organic matter content are the most important drivers of soil microbial biomass and activities (Craine et al., 2012). Fluctuations in climatic parameters such as warming or drought may have important consequences for microbial activities (Brinkman and Sombroek, 1996). Microbial biomass and activities are indicators of soil functioning because they are involved in the decomposition of soil organic matter and provisioning of nutrients controlling plant growth (Abrahão et al., 2022). The lower FDase activity may be related to the lower microbial biomass under panels, especially of gram-negative bacteria. Gram-negative bacteria are particularly sensitive to drought and lower temperatures. Bacteria interact with the soil mesofauna to recycle nutrients and sequester carbon (Basile-Doelsch et al., 2020), a key ecosystem service provided by semi-natural grasslands (Bengtsson et al., 2019). In our study, the lower abundance of mites and springtails under panels may be explained by the reduction of soil temperature but also by a lower plant biomass. Mesofaunal soil communities are particularly sensitive to temperature changes. Aupic-Samain et al. (2018, 2021) showed that abundance and diversity of the mesofauna organisms were lower in systems with lower temperatures. Moreover, the lower content in soil organic carbon under panels limits the growth of such microbial and mesofaunal decomposers.

4.3 Effects of solar panels on trophic interactions in the soil

Path analysis revealed either positive indirect (via organic carbon content) or negative direct effects of vegetation cover on soil mesofauna. An increase in plant cover increases the soil organic matter content, a food source for springtails that contribute to the microfragmentation of leaf litter (Berg and Laskowski, 2005). The negative direct effects of plant cover may be explained by a lower plant diversity in most productive plant communities. Zhang et al. (2022) reported in a metanalysis that soil fauna is most abundant and diverse in plant mixtures of high diversity through an increase in microhabitat complexity. Solar panels decreased plant diversity in our study and thus microhabitat diversity. In contrast, panels did not affect the positive indirect effect of vegetation cover on the abundance of mesofauna organisms. This result suggests that changes in microclimate under panels changed resource availability for such decomposers (Aupic-Samain et al., 2021).

In this study, we did not observe an effect of plant cover on microbial biomass and related interactions along the climatic along the climate gradient, neither outside nor under panels. However, plant litter is known to supply nutrients to soil microbes, and root exudates and rhizodeposition attract bacteria, fungi and nematodes (Chakraborty et al., 2012) suggesting that microbial communities are more sensitive to changes in climate than in plant cover. Changes in mean annual temperature and precipitation were the main drivers of bacterial biomass. Drenovsky et al. (2010) showed under a Mediterranean climate that land-use type and level of soil disturbance (i.e., tillage and irrigation regimes) are more important factors for the composition and biomass of the microbial community than vegetation.

We found more trophic interactions between panels compared to outside and under panels suggesting a protection effect of panels between rows on the soil food web. In the gap between panels, wind, albedo, soil temperature and solar radiation are generally lower than outside panels (Armstrong et al., 2014). Under panels, the effect of mean annual temperature and precipitation on trophic interactions diminished. The result may be explained by the homogenizing effect of panels on climate reducing maximum temperatures during daytime and summer and increasing minimum temperatures at night and during winter (Armstrong et al., 2016). Changes in precipitation and temperature are likely resulting in changes in the soil biodiversity and can alter soil biological processes and functions with potential consequences for ecosystem services (Pritchard, 2011; Nielsen and Ball, 2015). Soil CO_2 emission is an indicator of soil organic matter decomposition which integrates several biotic and abiotic components. CO_2 effluxes mainly depend on soil heterotrophic organisms (bacteria, fungi and fauna), plant roots (Bond-Lamberty et al., 2004; Moinet et al., 2019), soil temperature and moisture (Moinet et al., 2019). By changing plant communities, soil organisms and microclimate, the panels reduced direct and indirect effects of organisms in lower trophic levels, of mean annual temperature and of precipitation on CO_2 effluxes. Solar panels may thus limit ecosystem services such as carbon sequestration and nutrient cycling.

5 Conclusion

In solar parks, grassland communities were shaped by climatic factors such as semi-natural grasslands but solar panels partially disintegrate the relationship between climate and plant species composition. Solar panels changed plant communities towards more shade-tolerant species, reduced plant diversity and vegetation cover, and affected plant performance. Thus, solar parks allow the establishment of semi-natural grassland communities but this establishment is limited under solar panels. Changes in soil microclimate and lower plant productivity under panels negatively affected the abundance and composition of soil organisms, trophic interactions, and CO_2 effluxes. Ecosystem services provided by soil organisms such as carbon storage, nutrient regulation, and soil conservation, are thus hampered by solar panels. The ecological integration of solar parks to favour the establishment of semi-natural grasslands needs to limit the effect of panels on plant communities and soil quality, for example, by increasing inter-row space or panel height.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

QL, AB, and RG contributed to conception and design of the study. QL organized the database. QL, ME, AB, and RG contributed to fields sampling. QL, AB, and RG performed the statistical analysis. QL wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

Funding was provided by the French Agency for Environmental Transition (ADEME) via a PhD grant to Quentin Lambert and the project PIESO (agreement N°1405C0035).

Acknowledgments

We thank Pierre Illac and Marine David (TotalEnergie renouvelables), Julie pastor (EDF renouvelables), Marion Henriet (Cap Vert Energie), Jerôme Wampack (Cap Vert Energie), Alexandre Cluchier (ECO-MED) and Camille Rolin (Compagnie Nationale du Rhône) for their logistic support. We further thank Morgane Enea, Amélie Augier, Cassandra Favale, Léo Rocher, Lisa Foli,, Caroline Lecareux for assistance in the lab and in the field. We are particularly grateful to Daniel Pavon for his precious help in plant identification.

Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1137845/full#supplementary-material

References

Abrahão, A., Marhan, S., Boeddinghaus, R. S., Nawaz, A., Wubet, T., Hölzel, N., et al. (2022). Microbial drivers of plant richness and productivity in a grassland restoration experiment along a gradient of land-use intensity. *New Phytol.* 236, 1936–1950. doi:10.1111/nph.18503

Agati, G., Cerovic, Z. G., Pinelli, P., and Tattini, M. (2011). Light-induced accumulation of ortho-dihydroxylated flavonoids as non-destructively monitored by chlorophyll fluorescence excitation techniques. *Environ. Exp. Bot.* 73, 3–9. doi:10.1016/j.envexpbot.2010.10.002

Akinci, S. (2013). *Responses of organisms to water stress*. BoD – Books on Demand.

Armstrong, A., Waldron, S., Whitaker, J., and Ostle, N. J. (2014). Wind farm and solar park effects on plant–soil carbon cycling: uncertain impacts of changes in ground-level microclimate. *Glob. Change Biol.* 20, 1699–1706. doi:10.1111/gcb.12437

Armstrong, A., Ostle, N. J., and Whitaker, J. (2016). Solar park microclimate and vegetation management effects on grassland carbon cycling. *Environ. Res. Lett.* 11, 074016. doi:10.1088/1748-9326/11/7/074016

Aupic-Samain, A., Santonja, M., Chomel, M., Pereira, S., Quer, E., Lecareux, C., et al. (2021). Soil biota response to experimental rainfall reduction depends on the dominant tree species in mature northern Mediterranean forests. *Soil Biol. Biochem.* 154, 108122. doi:10.1016/j.soilbio.2020.108122

Aupic-Samain, A., Santonja, M., Forey, E., and Chauvat, M. (2018). Increasing temperature and decreasing specific leaf area amplify centipede predation impact on Collembola. *Eur. J. Soil Biol.* 89, 9–13. doi:10.1016/j.ejsobi.2018.08.002

Barnett, K. L., and Facey, S. L. (2016). Grasslands, invertebrates, and precipitation: A review of the effects of climate change. *Front. Plant Sci.* 7, 1196. doi:10.3389/fpls.2016.01196

Basile-Doelsch, I., Balesdent, J., and Pellerin, S. (2020). Reviews and syntheses: The mechanisms underlying carbon storage in soil. *Biogeosciences* 17, 5223–5242. doi:10.5194/bg-17-5223-2020

Bengtsson, J., Bullock, J. M., Egoh, B., Everson, C., Everson, T., O'Connor, T., et al. (2019). Grasslands—More important for ecosystem services than you might think. *Ecosphere* 10, e02582. doi:10.1002/ecs2.2582

Berg, B., and Laskowski, R. (2005). "Changes in substrate composition and rate-regulating factors during decomposition," in *Advances in ecological research* (Academic Press), 101–155. doi:10.1016/S0065-2504(05)38004-4

Bond-Lamberty, B., Wang, C., and Gower, S. T. (2004). A global relationship between the heterotrophic and autotrophic components of soil respiration? *Glob. Change Biol.* 10, 1756–1766. doi:10.1111/j.1365-2486.2004.00816.x

Boonman, C. C. F., Santini, L., Robroek, B. J. M., Hoeks, S., Kelderman, S., Dengler, J., et al. (2021). Plant functional and taxonomic diversity in European grasslands along climatic gradients. *J. Veg. Sci.* 32, e13027. doi:10.1111/jvs.13027

Brinkman, R., and Sombroek, W. G. (1996). "The effects of global change on soil conditions in relation to plant growth and food production," in *Global climate change and agricultural production*, 49–63.

Buyer, J. S., and Sasser, M. (2012). High throughput phospholipid fatty acid analysis of soils. *Appl. Soil Ecol.* 61, 127–130. doi:10.1016/j.apsoil.2012.06.005

Chakraborty, S., Pangga, I. B., and Roper, M. M. (2012). Climate change and multitrophic interactions in soil: The primacy of plants and functional domains. *Glob. Change Biol.* 18, 2111–2125. doi:10.1111/j.1365-2486.2012.02667.x

Chauvier, Y., Thuiller, W., Brun, P., Lavergne, S., Descombes, P., Karger, D. N., et al. (2021). Influence of climate, soil, and land cover on plant species distribution in the European Alps. *Ecol. Monogr.* 91, e01433. doi:10.1002/ecm.1433

Chen, M., Chory, J., and Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.* 38, 87–117. doi:10.1146/annurev.genet.38.072902.092259

Cherwin, K., and Knapp, A. (2012). Unexpected patterns of sensitivity to drought in three semi-arid grasslands. *Oecologia* 169, 845–852. doi:10.1007/s00442-011-2235-2

Choi, C. S., Cagle, A. E., Macknick, J., Bloom, D. E., Caplan, J. S., and Ravi, S. (2020). Effects of revegetation on soil physical and chemical properties in solar photovoltaic infrastructure. *Front. Environ. Sci.* 8. doi:10.3389/fenvs.2020.00140

Cleland, E. E., Collins, S. L., Dickson, T. L., Farrer, E. C., Gross, K. L., Gherardi, L. A., et al. (2013). Sensitivity of grassland plant community composition to spatial vs. temporal variation in precipitation. *Ecology* 94, 1687–1696. doi:10.1890/12-1006.1

Craine, J. M., Nippert, J. B., Elmore, A. J., Skibbe, A. M., Hutchinson, S. L., and Brunsell, N. A. (2012). Timing of climate variability and grassland productivity. *Proc. Natl. Acad. Sci.* 109, 3401–3405. doi:10.1073/pnas.1118438109

Dostalek, J., and Frantik, T. (2011). Response of dry grassland vegetation to fluctuations in weather conditions: A 9-year case study in prague (Czech republic). *Biologia* 66, 837–847. doi:10.2478/s11756-011-0079-1

Drenovsky, R. E., Steenwerth, K. L., Jackson, L. E., and Scow, K. M. (2010). Land use and climatic factors structure regional patterns in soil microbial communities. *Glob. Ecol. Biogeogr.* 19, 27–39. doi:10.1111/j.1466-8238.2009.00486.x

Ellenberg, H., Weber, H., Düll, R., Wirth, V., Erner, W., and Paulissen, D. (1992). Zeigwerte von Pflanzen in Mitteleuropa. *Scripta Geobotaníka-Gottingen*.

Fick, S. E., and Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37, 4302–4315. doi:10.1002/joc.5086

Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J., Attinger, S., et al. (2014). How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? *PLOS ONE* 9, e98987. doi:10.1371/journal.pone.0098987

Frostegård, Å., Tunlid, A., and Bååth, E. (1993). Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl. Environ. Microbiol.* 59, 3605–3617. doi:10.1128/aem.59.11.3605-3617.1993

Gang, C., Zhou, W., Chen, Y., Wang, Z., Sun, Z., Li, J., et al. (2014). Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. *Environ. Earth Sci.* 72, 4273–4282. doi:10.1007/s12665-014-3322-6

Green, V. S., Stott, D. E., and Diack, M. (2006). Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biol. Biochem.* 38, 693–701. doi:10.1016/j.soilbio.2005.06.020

Guénon, R., Day, T. A., Velazco-Ayuso, S., and Gros, R. (2017). Mixing of Aleppo pine and Holm oak litter increases biochemical diversity and alleviates N limitations of microbial activity. *Soil Bio. Biochemi.* 105, 216–226. doi:10.1016/j.soilbio.2016.11.023

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., and Wiezik, M. (2013). European grassland ecosystems: Threatened hotspots of biodiversity. *Biodivers. Conservation* 22, 2131–2138. doi:10.1007/s10531-013-0537-x

Hernandez, R. R., Armstrong, A., Burney, J., Ryan, G., Moore-O'Leary, K., Diédhiou, I., et al. (2019). Techno–ecological synergies of solar energy for global sustainability. *Nat. Sustain.* 2, 560–568. doi:10.1038/s41893-019-0309-z

Hernandez, R. R., Easter, S. B., Murphy-Mariscal, M. L., Maestre, F. T., Tavassoli, M., Allen, E. B., et al. (2014). Environmental impacts of utility-scale solar energy. *Renew. Sustain. Energy Rev.* 29, 766–779. doi:10.1016/j.rser.2013.08.041

Isbell, F., Adler, P. R., Eisenhauer, N., Fornara, D., Kimmel, K., Kremen, C., et al. (2017). Benefits of increasing plant diversity in sustainable agroecosystems. *J. Ecol.* 105, 871–879. doi:10.1111/1365-2745.12789

Julve, P. (1998). *Baseflor. Index botanique, écologique et chorologique de la flore de France*. Version: Oct 4th 2022.

Lambert, Q., Bischoff, A., Cueff, S., Cluchier, A., and Gros, R. (2021). Effects of solar park construction and solar panels on soil quality, microclimate, CO2 effluxes, and vegetation under a Mediterranean climate. *Land Degrad. Dev.* 32, 5190–5202. doi:10.1002/ldr.4101

Lambert, Q., Gros, R., and Bischoff, A. (2022). Ecological restoration of solar park plant communities and the effect of solar panels. *Ecol. Eng.* 182, 106722. doi:10.1016/j.ecoleng.2022.106722

Ma, Z., Li, S., Zhang, M., Jiang, S., and Xiao, Y. (2010). Light intensity affects growth, photosynthetic capability, and total flavonoid accumulation of anoectochilus plants. *HortScience* 45, 863–867. doi:10.21273/HORTSCI.45.6.863

Maestre, F. T., Callaway, R. M., Valladares, F., and Lortie, C. J. (2009). Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.* 97, 199–205. doi:10.1111/j.1365-2745.2008.01476.x

Malcolm, J. R., Liu, C., Neilson, R. P., Hansen, L., and Hannah, L. (2006). Global warming and extinctions of endemic species from biodiversity hotspots. *Conserv. Biol.* 20, 538–548. doi:10.1111/j.1523-1739.2006.00364.x

Moinet, G. Y. K., Midwood, A. J., Hunt, J. E., Rumpel, C., Millard, P., and Chabbi, A. (2019). Grassland management influences the response of soil respiration to drought. *Agron. Basel* 9, 124. doi:10.3390/agronomy9030124

Moradi, J., Mudrák, O., Kukla, J., Vicentini, F., Šimáčková, H., and Frouz, J. (2017). Variations in soil chemical properties, microbial biomass, and faunal populations as related to plant functional traits, patch types, and successional stages at Sokolov post-mining site - a case study. *Eur. J. Soil Biol.* 83, 58–64. doi:10.1016/j.ejsobi.2017.10.001

Moscatelli, M. C., Marabottini, R., Massaccesi, L., and Marinari, S. (2022). Soil properties changes after seven years of ground mounted photovoltaic panels in Central Italy coastal area. *Geoderma Reg.* 29, e00500. doi:10.1016/j.geodrs.2022.e00500

Nielsen, U. N., and Ball, B. A. (2015). Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. *Glob. Change Biol.* 21, 1407–1421. doi:10.1111/gcb.12789

Peterson, R. A. (2021). Finding optimal normalizing transformations via bestNormalize. *R J.* 13, 310. doi:10.32614/rj-2021-041

Pritchard, S. G. (2011). Soil organisms and global climate change. *Plant Pathol.* 60, 82–99. doi:10.1111/j.1365-3059.2010.02405.x

Pulungan, M. A., Suzuki, S., Gavina, M. K. A., Tubay, J. M., Ito, H., Nii, M., et al. (2019). Grazing enhances species diversity in grassland communities. *Sci. Rep.* 9, 11201. doi:10.1038/s41598-019-47635-1

Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *J. stati. software* 48, 1–36.

Steinauer, K., Tilman, D., Wragg, P. D., Cesarz, S., Cowles, J. M., Pritsch, K., et al. (2015). Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. *Ecology* 96, 99–112. doi:10.1890/14-0088.1

Uldrijan, D., Černý, M., and Winkler, J. (2022). Solar park – opportunity or threat for vegetation and ecosystem. *J. Ecol. Eng.* 23, 1–10. doi:10.12911/22998993/153456

Uldrijan, D., Kováčiková, M., Jakimiuk, A., Vaverková, M. D., and Winkler, J. (2021). Ecological effects of preferential vegetation composition developed on sites with photovoltaic power plants. *Ecol. Eng.* 168, 106274. doi:10.1016/j.ecoleng.2021.106274

van de Ven, D-J., Capellan-Peréz, I., Arto, I., Cazcarro, I., de Castro, C., Patel, P., et al. (2021). The potential land requirements and related land use change emissions of solar energy. *Sci. Rep.* 11, 2907. doi:10.1038/s41598-021-82042-5

Whiles, M. R., and Charlton, R. E. (2006). The ecological significance of tallgrass prairie arthropods. *Annu. Rev. Entomol.* 51, 387–412. doi:10.1146/annurev.ento.51.110104.151136

Wilson, J. B., Peet, R. K., Dengler, J., and Pärtel, M. (2012). Plant species richness: The world records. *J. Veg. Sci.* 23, 796–802. doi:10.1111/j.1654-1103.2012.01400.x

Xia, Y., and Yang, Y. (2019). RMSEA, CFI, and TLI in structural equation modeling with ordered categorical data: The story they tell depends on the estimation methods *Behav. Res. Methods* 51, 409–428. doi:10.3758/s13428-018-1055-2

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D., and Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: Are there any links? *Ecology* 84, 2042–2050. doi:10.1890/02-0433

Zarzycki, J., and Bedla, D. (2017). The influence of past land-use and environmental factors on grassland species diversity. *Appl. Ecol. Environ. Res.* 15, 267–278. doi:10.15666/aeer/1504_267278

Zhang, Y., Peng, S., Chen, X., and Chen, H. Y. H. (2022). Plant diversity increases the abundance and diversity of soil fauna: A meta-analysis. *Geoderma* 411, 115694. doi:10.1016/j.geoderma.2022.115694

frontiers | Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 26 July 2023
DOI 10.3389/fevo.2023.1136206

OPEN ACCESS

EDITED BY
Zoltán Bátori,
University of Szeged, Hungary

REVIEWED BY
Karel Prach,
University of South Bohemia in České Budějovice, Czechia
László Erdős,
Hungarian Academy of Science, Hungary

*CORRESPONDENCE
Grégory Mahy
✉ g.mahy@uliege.be

RECEIVED 02 January 2023
ACCEPTED 28 April 2023
PUBLISHED 26 July 2023

CITATION
Dellicour M, Goret T, Piqueray J, Fayolle A, Bindelle J and Mahy G (2023) Success of passive and active restoration of lowland hay meadows with regard to current and historical references.
Front. Ecol. Evol. 11:1136206.
doi: 10.3389/fevo.2023.1136206

Success of passive and active restoration of lowland hay meadows with regard to current and historical references

Maïké Dellicour[1], Thibaut Goret[2], Julien Piqueray[3], Adeline Fayolle[1], Jérôme Bindelle[1] and Grégory Mahy[1,4]*

[1]Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium, [2]Natagora, Traverse des Muses, Namur, Belgium, [3]Natagriwal ASBL, Gembloux, Belgium, [4]Avignon Univ, Aix Marseille Univ, CNRS, IRD, Avignon, France

The restoration of hay meadows is one of the major challenges in temperate Europe, to overcome the drastic loss and degradation of these semi-natural habitats. Evaluation of restoration success requires the definition of a relevant reference ecosystem. Historical phytosociological relevés dating from the 1960s–1970s, before large scale agriculture intensification, may be used as the reference framework for such purposes. However, this reference framework may no longer be pertinent in our present-day ecological and socio-economic context. In this study, we assessed the success of three restoration techniques for lowland hay meadows, based on plant species richness and composition and functional traits, by comparing the restoration trajectories to historical and current references. The three restoration techniques, passive restoration mowing, active fresh hay transfer and active sowing of threshing material, were implemented depending on the initial level of degradation and the proximity to well-preserved meadows. The species composition of restored meadows clearly differed from historical references but converged toward current references, for both passive and active restoration techniques. Historical references are characterized by short, stress-tolerant dicotyledonous plant species with small specific leaf area, indicators of nutrient-poor environments. The gap observed between plant communities of historical and current meadows suggests that the historical data are an over-ambitious target for restoration purposes and can hardly be considered relevant to our present-day context. Therefore, creation of a new reference framework of lowland hay meadows is needed for the adequate implementation of restoration projects. Successful outcomes of active and passive techniques must encourage practitioners to adapt the choice of the restoration method depending on the local ecological context, i.e., level of degradation and proximity to well-preserved habitats.

KEYWORDS

grassland, restoration success, diaspore transfer, restoration reference, plant community, hay transfer, mowing

1. Introduction

Temperate meadows are among the habitats that host the most diverse fauna and flora in temperate Western Europe (Veen et al., 2009; Bruchmann and Hobohm, 2010; Wilson et al., 2012; Habel et al., 2013). The high conservation value of these habitats is the result of centuries, even millennia, of extensive traditional management allowing the coexistence of many species in these open environments (Isselstein et al., 2005). Since the second half of the twentieth century, changes in agricultural practices were encouraged by the implementation of the European Common Agricultural Policy (CAP). Land use changes and intensification of agricultural systems have led to the decline and degradation of species-rich meadows (Stoate et al., 2009; Veen et al., 2009), and therefore to a significant loss of animal and plant biodiversity in rural landscapes (Zechmeister et al., 2003; Marini et al., 2008; Manning et al., 2015; Emmerson et al., 2016). Specifically, lowland hay meadows are assessed as being in a poor conservation state over almost all of their range in the EU, which extends from northern Portugal to southern Sweden with a high occurrence in Belgium and Germany (European Environment Agency, 2019, 2020). Hence, ecological restoration of hay meadows is a major challenge in temperate Europe.

The definition of a clear and relevant reference ecological system is a central question for the evaluation of restoration success (Holl and Cairns, 2002; Waldén and Lindborg, 2016; Prach et al., 2019). Reference information is usually either existing intact sites or historical data (White and Walker, 1997). In Europe, a large dataset of phytosociological relevés have been collected historically (1960s–70s). Those relevés, providing information on plant communities before the major effect of agriculture intensification, are often used as references for evaluating the conservation status and restoration success of ecosystems. In southern Belgium, they have been used to build the regional version of EUNIS habitat classification (Davies et al., 2004) and are considered to be the reference framework for evaluating the outcome of EU-funded restoration programs targeting ecosystem restoration (Dufrêne and Delescaille, 2005). However, this historical reference may no longer be achievable in our present-day ecological and socio-economic context. Rapid global environmental changes, mostly climate change and global eutrophication (Matson et al., 2002), may impose the reconsideration of the use of historical data as a reference for ecological restoration (Jackson and Hobbs, 2009; Higgs and Jackson, 2017). When choosing the reference data for the restoration of agriculture-dependent ecosystems, the evolution of agro-ecosystem management driven by socio-economic factors must also be taken into consideration as they greatly influence farmers' practices (McGinlay et al., 2017). In this context, former reference communities imposing very specific species assemblages may be an over-ambitious target. Despite this question of the use of historical references for evaluating the restoration of agro-ecosystems, only a small number of studies compared historical and contemporary references for evaluating large-scale, ecological restoration projects (Barak et al., 2015).

A variety of restoration techniques, including active and passive methods, are commonly used to overcome abiotic, biotic and dispersal constraints in meadow restoration projects (Sengl et al., 2017). Hay meadows have been historically managed by mowing with low fertilization. Adaptation of key management practices (fertilization, mowing regime) is seen as a passive way to restore meadows (Jones et al., 2018). Passive restoration is recommended when the habitat is slightly degraded, has maintained a great potential for natural self-repairing and/or is integrated into a propitious landscape context, i.e., when surrounded by less impaired and traditionally managed meadows in which target species remain common (Jones et al., 2018; Prach et al., 2020). In more degraded landscape contexts and more strongly altered ecosystems, modification of management alone is insufficient for restoration. Lack of dispersal of the target species in time or space, i.e., the dispersal constraint is often identified as a major barrier (Donath et al., 2003; Hölzel et al., 2012; Pywell et al., 2012; Kiss et al., 2016). In such contexts, active restoration with assisted species dispersal combined with appropriate management practices is needed.

Plant assemblages are traditionally studied at the species taxonomic level, which allows for direct reference to a target species assemblage. Using predictions based on the taxonomic level alone may not allow the detection of functional differences, and the results obtained from a list of species in a given context cannot be extended to all contexts. Trait-based approaches are complementary to help to identify functional patterns and ecological functions independently of the studied pool of species (Lavorel and Garnier, 2002; Kahmen and Poschlod, 2004). Functional traits are useful tools to understand responses to environmental changes, such as changes in nutrient availability and disturbances (Lavorel and Garnier, 2002). As these abiotic conditions are expected to change during meadow restoration and between historical and current communities, we can also expect to see changes in functional composition (Fagan et al., 2008).

In this study, we aimed to assess the success of passive (mowing regime) and active (transfer of fresh seed-containing hay and sowing of threshing material) restoration of lowland hay meadows by confronting the restoration trajectories to historical and current references. We use an *in-situ* large scale experiment situation provided by the LIFE project in southern Belgium. We specifically addressed the following questions:

(1) Does the restoration of degraded meadows correspond to the recovery of the species and functional composition of historical and/or current references?
(2) Do passive and active restoration efforts, that were implemented depending on the initial level of meadow degradation, lead to equivalent restoration success?

2. Materials and methods

2.1. The restoration project

In southern Belgium, the ecological situation of lowland hay meadows is particularly unfavorable (Wibail et al., 2014; DEMNA, 2019). Several EU-funded LIFE projects have been launched to restore hundreds of hectares of lowland hay meadows using a variety of empirical techniques and offering great *in situ* experiments to evaluate restoration success. This study focused on the LIFE project "Prairies bocagères" (LIFE11 NAT/BE/001059; Goret et al., 2020) in which the restoration of approximately 200 hectares of lowland hay meadows was carried out in the Fagne-Famenne bioclimatic region, which is characterized by Upper Devonian shale geology and loam-rocky to clay soils. The mean annual temperature is 9.5°C and the mean annual precipitation is 976 mm (Van der Perre et al., 2015). Restoration actions were implemented in seven nature reserves with meadows presenting different levels of degradation (Figure 1). A large number of degraded meadows were included in the restoration process each year from 2013 to 2018. This chronosequence of restoration is of significant value as it allows us to analyse changes in community composition over time (Engst et al., 2017; Sullivan et al., 2020).

The habitat targeted by restoration is the Natura 2000 habitat 6,510 protected by the EU Habitats Directive 92/43/EEC (European Community, 1992 – Annex I "Lowland hay meadows"; code EUNIS E2.22; phytosociology *Arrhenatherion elatioris* Koch 1926). *Arrhenatherion* hay meadows are permanent grasslands found on mesic soils at low altitudes (<300 m in Belgium). They are first mowed in early summer (end of June to early July). Depending on the meadow productivity and the yearly weather conditions, a second mowing or low-intensity grazing can occur at the end of summer. No or low fertilization is applied. When in good conservation status, they are characterized by oligo- to mesotrophic plant species (see lists 1 and 2, Supplementary Table S1).

The LIFE project implemented three restoration techniques: passive restoration mowing, active restoration with either transfer of hay containing fresh seed or direct sowing of threshing material. The restoration technique was adapted depending on the initial degradation level and the direct vicinity of the meadow following the decision-making tool of Goret et al. (2021). The techniques used depended on the initial conservation status (CS) of the meadow to be restored (Supplementary Table S2). CS was determined using indicators of plant community integrity following the evaluation grid of the Department for Nature and Agriculture Study of the Regional Administration (Supplementary Table S1; A: very good CS; B: good to medium CS; C: degraded CS; D: not a lowland hay meadow).

A total of 42 restored parcels were selected for this study. If the CS corresponded to recent degradation (B or C for less than 5 years) or a degraded meadow (C) with an adjacent seed source (meadow with very good CS-A), the strategy was to use mowing (passive restoration), cutting and exporting biomass twice a year: once after June 15 and once after September 15. The first mowing was delayed by about a month compared to the regime applied before the restoration (May 15). During the first years of restoration, the first mowing cannot be too late, even though this is recommended to allow all species to complete their life cycle, because abiotic conditions first need to be adequate to favor forb germination and development. Mowing in mid-June helps to maximize nutrient export and to create sites where reduced competition for light allows forb seedlings to establish. Fertilization was proscribed.

If the CS was C (degraded) for more than 5 years with no adjacent grassland in CS-A or if CS was D, the restoration strategy included: stopping fertilization, soil preparation for seedling establishment (creation of bare soil strips by two harrow operations or by milling to a depth of 10 cm), assisted seed dispersal by fresh hay transfer or sowing of threshing material, and a post-restoration mowing regime similar to passive restoration. For both assisted seed dispersal methods, plant material was collected directly from a donor site in CS-A, located as close as possible (less than 10 km). Threshing material and fresh hay were always collected at the end of July, the year of restoration, in order to maximize the amount of characteristic species that had reached seed-maturity. Threshing material was collected using a small combine harvester adjusted to limit the amount of plant residues garnered. The harvest product was dried for about 48 h and then roughly sorted using an alveolar pre-sorter in order to not lose seed diversity. Through this process, less than 10% of plant debris remained in the seed mixture. In September, 25 kg per hectare was sown with a mechanical seeder in bare soil strips. For fresh hay transfer, the hay was spread on the degraded site on the same day that the donor site was mowed.

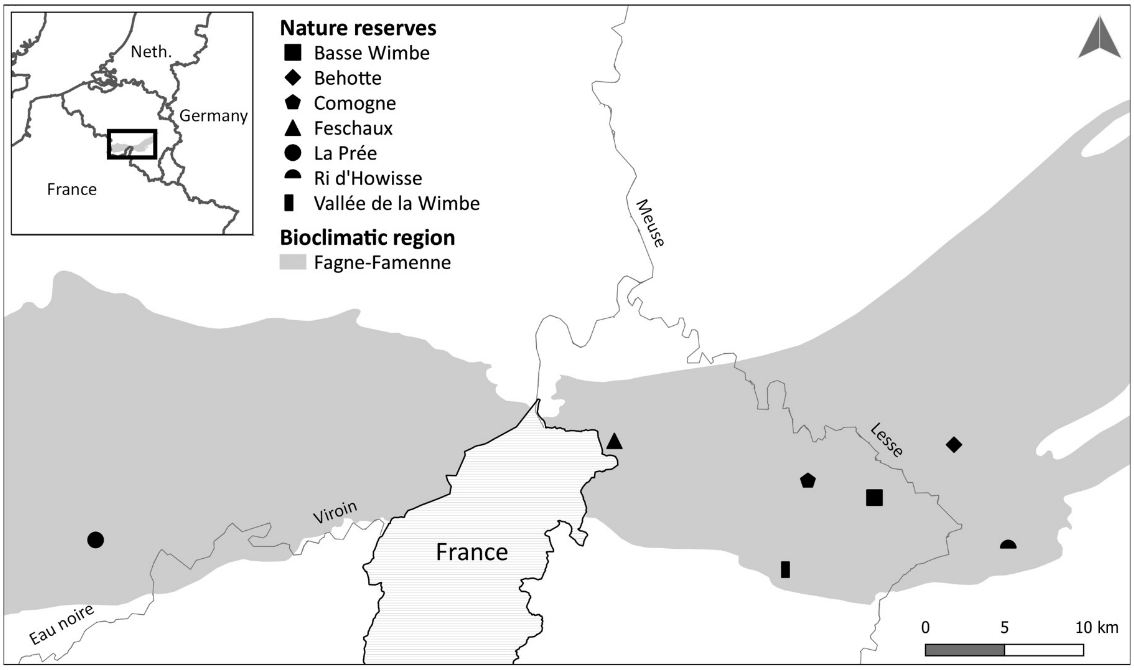


FIGURE 1
Location of the Fagne-Famenne region and the seven locations where meadow restoration occurred in the vicinity of reference nature reserves.

TABLE 1 Number of meadow parcels per restoration technique and years since restoration.

	1–2 years (2017–18)	3–4 years (2015–16)	5–6 years (2013–14)
Restoration mowing	4	6	4
Transfer of fresh seed-containing hay	/	6	6
Threshing material sowing	5	6	5

TABLE 2 Hypotheses defining expected changes in plant community traits of restored meadows.

Attributes	Hypotheses
Leaf dry matter content (LDMC)	Increases with soil nutrient depletion (Lavorel et al., 2007). A high LDMC reflects a resource conservation strategy that should prevail in reference and restored ecosystems (Lavorel and Garnier, 2002)
Specific leaf area (SLA)	Decreases with soil nutrient depletion (Lavorel et al., 2007). A low SLA reflects a resource conservation strategy that should prevail in reference and restored ecosystems (Lavorel and Garnier, 2002)
Height	Decreases with soil nutrient depletion and thus with the reduction of competition for light that should prevail in reference and restored ecosystems (Hautier et al., 2009)
Raunkiaer's biological types distribution	Proportion of therophytes increases with restoration due to adaptation of the mowing regime
Life form distribution	Proportion of grasses decreases as proportion of dicotyledonous and legume plants increases with restoration
Ellenberg's N index	Decreases with soil nutrient depletion which should prevail in reference and restored ecosystems
CSR strategy distribution	Proportion of competitive and ruderal plants decreases while proportion of stress-tolerant plants increases during restoration (Walker et al., 2004)

2.2. Vegetation sampling

A space-for-time substitution approach was used to characterize the restoration trajectory based on plant communities. In 2019, we chose meadows that have been restored for one to 6 years (restoration age) with an equal repartition among three age classes since restoration: 1–2 years, 3–4 years and 5–6 years (Table 1). Altogether, the restored meadows covered a total of 58.6 ha.

A complete botanical survey of each meadow was undertaken before the restoration (initial state) and once at the end of the project in 2019 (restored state). These surveys were conducted from mid-May to the end of June and were carried out by walking the entire meadow, avoiding edges. The cover of all herbaceous species encountered at the parcel scale was visually estimated using the Braun-Blanquet scale (r = one individual, + = <1%, 1 = 1–5%, 2 = 5–25%, 3 = 25–50%, 4 = 50–75%, 5 = >75%). Braun-Blanquet scale was chosen to use the same method and precision of abundance estimation as historical references. The list was considered exhaustive if no other species was found after a walk of approximately 10 min in the meadow. The nomenclature used in this study follows the flora of Lambinon and Verloove (2015).

2.3. Reference data

Community recovery trajectories were evaluated against two types of reference data representing *Arrhenatherion* meadows in favorable CS: (1) six meadows in nature reserves of Fagne-Famenne region that were meadows in the best conservation status in the region (as defined in Supplementary Table S1; hereafter current references). Floristic composition of the six current references was surveyed following the same protocol as described above for restored parcels and (2) 36 historical surveys recorded from 1955 to 1963, and one recorded in 1996, in the Fagne-Famenne region (hereafter historical references). Historical surveys were taken from the data used for the phytosociological description of the EUNIS typology in southern Belgium which served to define the very good conservation state of meadows (CS-A). For the historical references, one relevé per meadow was realized with sampling area varying from 50 to 200 m^2.

2.4. Functional traits

Seven functional traits were retained to characterize the functional differences between reference, restored and degraded plant communities. Each trait was selected because it is expected to change with restoration according to predetermined hypotheses (Table 2).

Information on leaf attributes (leaf dry matter content LDMC and specific leaf area SLA) and canopy height of species was extracted from the LEDA database (Kleyer et al., 2008). For traits where several values per species were given, the average of the values from countries close to Belgium (Germany, Netherlands, United Kingdom, France) was calculated. If no height data was provided for a species, the maximum height given in the flora of Lambinon and Verloove (2015) was used. Raunkiaer's biological types were taken from Lambinon and Verloove (2015). When two biological types were mentioned, the first cited was retained. Life forms (grass, legume and other dicotyledonous plant) were also included in the analysis. The N-Ellenberg's index classifying species according to their nutrient requirements was taken from the Baseflor database (Julve, 1998). Grime's CSR values of each survey were calculated using the electronic spreadsheet-based tool of Hunt et al. (2004) which relies on the CSR types of 1,000 European species identified by Hodgson et al. (1999). The calculation of CSR positions in this spreadsheet is based on the relative abundance of species in the survey. If a species was not present in the spreadsheet, the CSR type in the BiolFlor database (Kühn and Klotz, 2002) was used.

For the analysis of changes in plant community functional traits between the historical reference and the current system (current reference and restored meadows), we proceeded to the following calculations. (1) Nominal traits (Raunkiaer's biological types and life forms) were transformed to as many binary traits as there were values for the trait. We then computed trait relative abundance as the proportion of total cover occupied by species sharing the trait. Only therophyte and hemicryptophyte were retained as binary traits for Raunkiaer's biological types as other types were too sparse in this habitat and (2) For numeric attributes (LDMC, SLA, canopy height, Ellenberg index), we calculated the community weighted mean (CWM; Violle et al., 2007; Lavorel et al., 2008) taking species cover as weight. Cover values were derived from the van der Maarel coefficients using the median cover (Supplementary Table S3). CWM of LDMC was only computed for grass species (hereafter LDMCgrass) in order to avoid effects due to changes in the proportion of life forms; the different life forms have contrasting ranges of LDMC (Cruz et al., 2002; Al Haj Khaled et al., 2005). Before any further calculations, the square root transformation was applied to variables when necessary in order to improve normality. This was done for the relative abundances of legumes, hemicryptophytes and therophytes, and S and R values.

2.5. Statistical analysis

Prior to data analysis, the Braun-Blanquet scale was transformed into van der Maarel (1979; Supplementary Table S3). All analyses were conducted using R statistical software (R Core Team, 2021).

To determine whether the restoration of degraded meadows corresponds to the recovery of the species composition of historical and/or current references, we used a principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity (Legendre and Legendre, 2012). In order to visualize the global trajectory and to explore the variation in species composition between historical and current references, the PCoA was first run using the entire dataset (historical references included). Because we observed differences between historical references and other situations, we used the multipatt() function of the "indicspecies" package with the IndVal index to identify species characteristics of the historical reference, current references and restored meadows (func = "IndVal.g"; De Cáceres et al., 2010; De Cáceres, 2020). This function was used because it allows the identification of indicator species of combinations of groups.

Because historical references were found to be different from current references in their species and functional composition, we focused the analysis of restoration trajectories on current references. A PCoA was performed on a restricted dataset excluding the historical references but with current references, in order to better examine variation in species composition between the initial and final states of restored parcels. The latter allowed the restoration trajectory of each meadow and technique-dependent trajectories to be visualized. PCoA were performed using the "vegan" package (Oksanen et al., 2020) and the cmdscale() command in the "stats" package.

Species richness of each survey was calculated as the number of observed species. In order to evaluate whether active and passive restoration led to changes in species composition, similarity with current references was calculated for all initial states and restored states at different periods. We first calculated the average current reference by computing the mean of species abundance across the six current references, and then we computed the Bray-Curtis distance between all restored sites and this average reference. Similarity was calculated as 1 Bray-Curtis distance. To determine whether species richness and similarity significantly increase with the age of restoration, linear regressions were computed separately for the three restoration techniques. To test for significant differences in slopes and intercepts, analysis of covariance (ANCOVA) was performed using the anova_test() command in the "rstatix" package (Kassambara, 2021) with species richness or similarity as the dependent variable, restoration technique as the grouping variable and restoration age as the covariate. *Post-hoc* tests were carried out using the emmeans_test() command of the "rstatix" package with a Bonferroni correction for multiple comparisons. To examine whether species richness of 5–6 year old restored parcels was similar to that of current references, analysis of variance (ANOVA) followed by a Dunnett test was performed. The glht() function of the "multcomp" package (Hothorn et al., 2021) was used for the Dunnett test. To evaluate whether the species composition of restored parcels was similar to that of current references, we compared similarities between restorations and current references with similarity found within current targets. Plots were visualized using the "ggplot2" package (Wickham et al., 2020).

3. Results

3.1. Restoration success against historical targets

The first two dimensions of the PCoA performed on the entire dataset (historical references included) explained 18.22% of the existing floristic variation (12.92 and 5.30%, respectively for the first two PCoA axes; Figure 2). The third dimension explained 4.69% of the variation but did not show any meaningful pattern. The species composition of the historical references (HR) clearly differed from all other floristic surveys (the six current references (CR), the initial state of restored meadows, the final states of restored meadows whatever the age of restoration), with the historical references exclusively positioned on the negative values of the first axis and others mainly situated on the positive values. The ordination on the second axis revealed a global trajectory where the plant communities of restored meadows become more distant from their initial degraded states over time. Correlations between plant attributes and PCoA scores showed that plant communities of historical references were more stress-tolerant (S) and had a higher proportion of dicotyledonous and legume species compared to current reference and restored parcels (Figure 2). In contrast, current plant communities (reference and restoration parcels) had a higher proportion of grasses and had a more competitive (C) and eutrophic (Ellenberg N) character. They were also characterized by a higher SLA and height. Plant communities of initial degraded states in restored meadows presented a higher ruderal strategy (R) and were more tolerant of frequent disturbances, showing the effect of the intensive mowing regime on degraded meadows. Initial states of restored meadows had a higher proportion of hemicryptophytes compared to final states of restoration and current references. Restored plant communities had a higher proportion of therophytes, dicotyledonous and legume species and a higher LDMCgrass.

We found seven significant exclusive indicator species of historical references that were not found in current references nor in restored meadows, and we identified eight other significant exclusive indicator species of historical references that were found at low frequency in current references and in restored meadows (Figure 3). Moreover, four

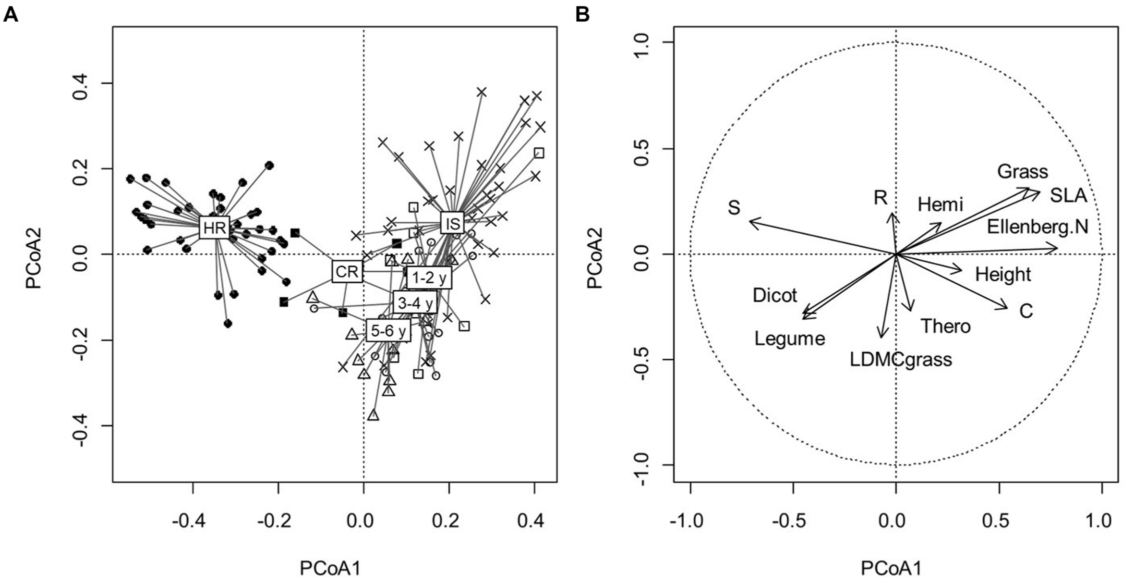


FIGURE 2
(A) Principal coordinate analysis (PCoA) performed on the 127 floristic surveys: × IS: initial states of restored parcels (*n*=42), □ final states for 1–2-year-old restoration (*n*=9), ○ final states for 3–4-year-old restoration (*n*=18), △ final states for 5–6-year-old restoration (*n*=15), ■ CR: current references (*n*=6), ● HR: historical references (*n*=37). The first two axes explained 18.22% of the floristic variation (PCoA1: 12.92%, PCoA2: 5.30%). **(B)** Correlation circle showing relations between PCoA scores and community plant functional traits.

species from the list of rare, threatened and protected spermatophyte species of the Walloon Region (Saintenoy-Simon et al., 2006) were only found in a very small number of historical references (*Alchemilla filicaulis*, *Alchemilla glaucescens*, *Festuca ovina*, *Ophioglossum vulgatum*). Inversely, nine significant exclusive indicators of current references were never found in historical references (e.g., *Myosotis arvensis*, *Elymus repens*). The complete list of all recorded species is provided in the Supplementary Table S4 as well as the value of p associated with their indicator status and their frequency in each group.

3.2. Recovery of species richness and species composition against current references

Species richness significantly increased with time for the three restoration techniques (mowing: $F = 13.94$, $p < 0.001$, threshing material sowing: $F = 10.49$, $p = 0.003$, and hay transfer: $F = 19.1$, $p < 0.001$; Figure 4). The slopes of the three regression lines were not significantly different according to the ANCOVA ($p = 0.972$) and each restoration trajectory gained on average 2.4 species per year. However, intercepts were significantly different ($p = 0.003$), in line with the fact that initial states differed among the meadows restored with the different techniques. *Post-hoc* tests showed that initial species richness of parcels restored with fresh hay transfer was significantly lower than that of parcels restored only by mowing (p-ajd = 0.002) but was not significantly different from that of parcels restored by threshing material sowing (p-adj = 0.190). The initial species richness was not significantly different for the latter two (p-ajd = 0.207). ANOVA and Dunnett tests revealed a significantly lower final species richness of parcels restored with fresh hay transfer (5–6 years after restoration) compared to species richness of current references. Other restoration methods led to similar species richness to current references (46.7 species) with for mowing: 45.5 species, $t = -0.264$, $p = 0.988$, threshing material sowing: 36.4 species, $t = -2.479$, $p = 0.062$, and fresh hay transfer: 33.5 species, $t = -3.335$, $p = 0.011$.

Similarity of restored plant communities to the average species composition of current references significantly increased with time from restoration for the three restoration techniques (mowing: $F = 12.18$, $p = 0.002$, threshing material sowing: $F = 25.86$, $p < 0.001$, and hay transfer: $F = 10.17$, $p = 0.004$; Figure 5). The slopes and intercepts of the three regression lines were not significantly different from one another (slopes: $p = 0.605$, intercepts: $p = 0.803$). Each restoration trajectory gained on average 2.37% similarity to current references per year. Similarity between final states of restored plant communities and current references was close to the mean similarity observed among current references (mean similarity among current references: 0.52, mean similarity between final states and current references: 0.51 for mowing, 0.50 for fresh hay transfer, 0.52 for sowing; Figure 5). This result indicates restoration success since the recovery of species composition attained the level of similarity found within references.

3.3. Restoration trajectories for restoration methods

The first two axes of the ordination performed on the restricted dataset excluding the historical references explained 16.55% of the floristic variation (PCoA1: 9.93%, PCoA2: 6.62%; Figure 6). In order to visualize the parcels' individual trajectories, each initial state was linked to its final state. Most of the individual trajectories converged towards current references, even when species composition was initially far from that of current references. In some cases, final states reached current references (Figure 6A). The initial states of the restored meadows covered a wide range of floristic variation, which is in accordance with

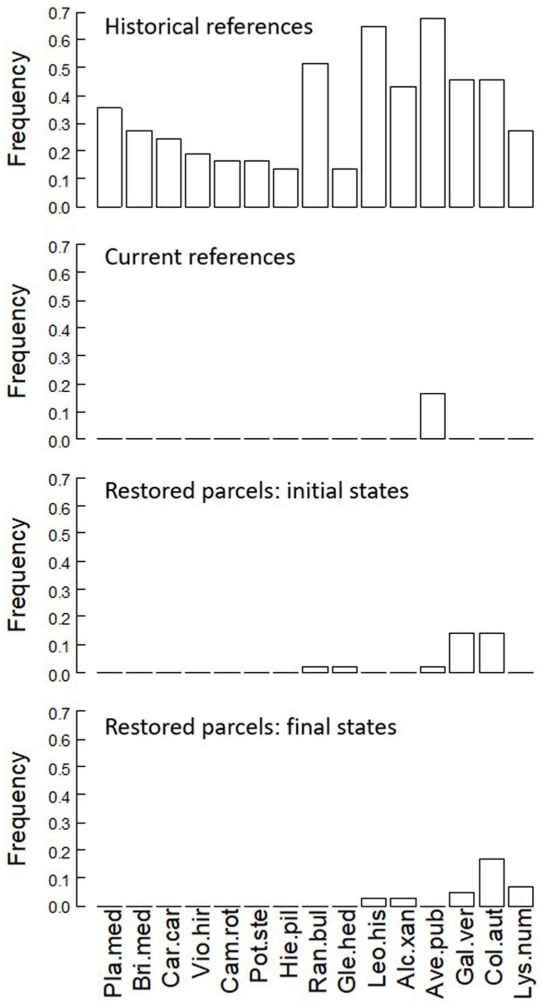


FIGURE 3
Frequency of significant exclusive indicator species (identified by the multipatt function) observed in historical references, current references, initial and final states of restored meadows. Species abbreviations: Pla.med, *Plantago media*; Bri.med, *Briza media*; Car.car, *Carex caryophyllea*; Vio.hir, *Viola hirta*; Pot.ste, *Potentilla sterilis*; Hie.pil, *Hieracium pilosella*; Ran.bul, *Ranunculus bulbosus*; Gle.hed, *Glechoma hederacea*; Leo.his, *Leotondon hispidus*; Alc.xan, *Alchemilla xanthochlora*; Ave.pub, *Avenula pubescens*; Gal.ver, *Galium verum*; Col.aut, *Colchicum autumnale*; Lys.num, *Lysimachia nummularia*.

the different initial conservation status for meadows restored with passive (mowing) and active (hay transfer and sowing) restoration techniques. However, most of them differed from the other surveys in their position on the positive values of the first axis (Figure 6A).

In order to examine technique-dependent ecological trajectories, this PCoA was then presented with a dissociation of the initial and final states of the three restoration techniques (Figure 6B). This representation allowed clear differences to be observed between the initial states (IS) of communities restored by the different restoration techniques. Initial states of meadows restored by transfer of fresh seed-containing hay (Ha) and by threshing material sowing (So) were clearly more distant from current references than those of meadows restored by restoration mowing (Mo). This representation also showed that, regardless of the restoration technique, the final states (FS) of plant communities converged in the direction of current references.

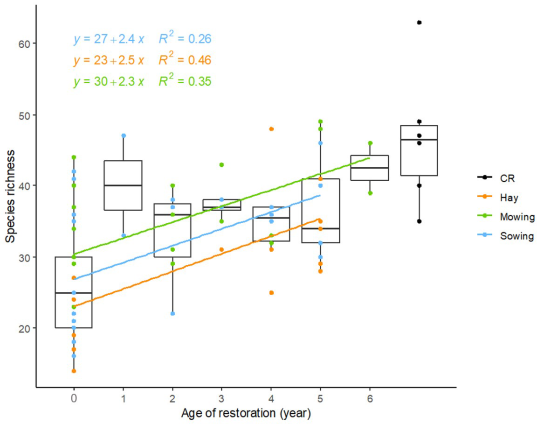


FIGURE 4
Box-plots of species richness variation for initial state of restored meadows (year 0), species richness of restored meadows at different restoration ages and current references (CR). Lines represent linear regression models between restored meadow species richness and time since restoration began for the three restoration methods. Hay: fresh hay transfer, Sowing: sowing of threshing material, Mowing: passive restoration by mowing regime adaptation (Mowing: F=13.94, p<0.001, Sowing: F=10.49, p=0.003, Hay: F=19.1, p<0.001).

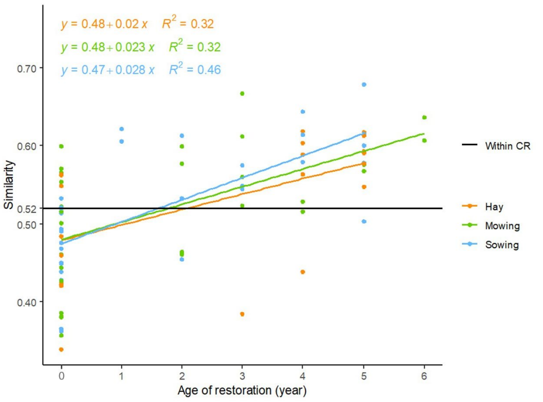


FIGURE 5
Linear regression models showing significant relationships between species composition similarity (1 Bray-Curtis distance) with the average species composition of current references (CR) and time since restoration began for the three restoration methods. Hay: fresh hay transfer, Sowing: sowing of threshing material, Mowing: passive restoration by mowing regime adaptation (Mowing: F=12.18, p=0.002, Sowing: F=25.86, p<0.001, Hay: F=10.17, p=0.004). Within CR=average similarity within current references. Slopes and intercepts of the three regression lines are not significantly different (slopes: p=0.605, intercepts: p=0.803).

4. Discussion

Our results highlight a strong floristic difference between historical references, based on historical phytosociological data (1960s–70s) of lowland meadows, and current references based on existing meadows with the best conservation status in the studied region, toward which the final states of restoration converged. Historical meadows differed from current references and restored meadows by being mostly short, stress-tolerant dicotyledonous plant communities with small SLA. Such

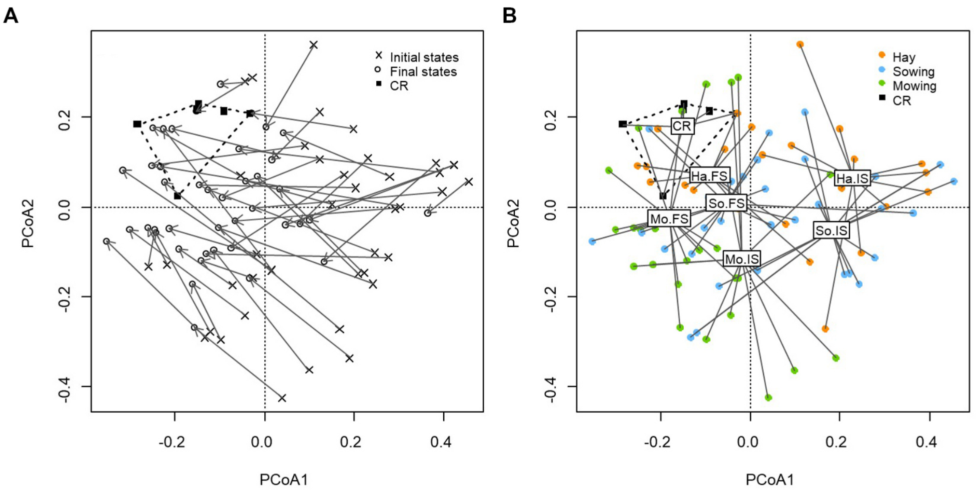


FIGURE 6
Principal coordinate analysis (PCoA) performed on species composition for restored meadows and current references. The first two axes explained 16.55% of the floristic variation (PCoA1: 9.93%, PCoA2: 6.62%). **(A)** Arrows link the initial state and final state of each restored meadow to show individual ecological trajectories. Initial states (*n*=42), final states (*n*=42), current target references (*n*=6). **(B)** PCoA with dissociation of the initial and final states of the three restoration techniques to display technique-dependent ecological trajectories. Mo.IS: initial states of mowing (*n*=14), Mo.FS: final states of mowing (*n*=14), Ha. IS: initial state of fresh hay transfer (*n*=12), Ha.FS: final state of fresh hay transfer (*n*=12), So.IS: initial state of sowing (*n*=16), So.FS: final state of sowing (*n*=16), current reference targets (*n*=6).

a functional composition indicates a prevailing resource conservation strategy in historical references compared to restored meadows and current references. This was confirmed by the detection of indicator species of historical references that are known to grow in low nutrient environments. In contrast, restored meadows and current references were characterized by a more important contribution of tall, long-lived and competitive graminoids with high SLA, and a set of indicator species that preferentially develop in more nutrient-rich environments (e.g., *Elymus repens, Geranium pyrenaicum, Lapsana communis*). Those results provide relevant information about community changes between the historical and contemporary states of ecosystems (Barak et al., 2015; Higgs and Jackson, 2017) and show that significant changes occurred over a time span of *ca.* 65 years (between 1955 and 2020). This pattern is in accordance with overall plant community changes induced by anthropogenic drivers (Timmermann et al., 2015), specifically nitrogen deposition induced by anthropogenic activities in the 20th century, which is recognized as one of the major threats to plant biodiversity in European herbaceous ecosystems (Stevens et al., 2004; Bobbink et al., 2010; Stevens et al., 2018).

The gap observed between historical and current meadows with very good conservation status suggests that the historical data based on phytosociological surveys dating from ~60 years ago, and being the basis for the European habitat typology in Belgium, may be an over-ambitious target for restoration purposes in the current global environmental and socio-economic context. As often mentioned in the ecological restoration literature, the desire to reach, at all costs, a static reference state of the past in the current context of rapid and unprecedented global changes is most likely to be in vain (Harris et al., 2006; Choi et al., 2008; Allison, 2017). Achievable and sustainable outcomes cannot be reached with a target that may satisfy a nostalgic need but is inconsistent with the future (Harris et al., 2006). The expected future reduction in nitrogen deposition is unlikely to induce significant species recovery (Payne et al., 2017). In addition to historical global eutrophication which has already impacted grassland ecosystems in the temperate regions, climate change and increasingly frequent climate extremes are expected to cause long-term shifts in plant community composition (Gornish and Tylianakis, 2013; Shi et al., 2015). Also, societal expectations may prevent the return to the historical reference because it no longer matches a socially and economically acceptable goal for agriculture-dependent meadows (Choi, 2007; McNellie et al., 2020). For these reasons, history should not serve as a target but rather as a guide for restoration (Harris et al., 2006; Higgs et al., 2014). Achieving a specific taxon-based assemblage is no longer absolutely sought, but rather a set of functions, ecological processes and ecosystem services that restore ecosystem health (SER, 2004; Choi et al., 2008) and allow biodiversity restoration on a larger scale than that of the phytosociological survey, i.e., at the landscape or ecosystem scale (Choi, 2007). In the same line, the historical targets included plant community composition described by surveys following the Zurich-Montpellier method, i.e., a non-random and subjective sampling method where the most typical and representative sampling points of the studied phytosociological association were used (Becking, 1957). They are therefore the most biologically valuable representation of the *Arrhenatherion* association at the time, which has probably accentuated the contrast observed with current floristic communities. In addition, the number of current references was limited in this study because of the restricted availability of such meadows in current European temperate agricultural landscapes. Thus, there is an important need to create a well-defined reference framework of lowland hay meadows for the different essential steps of a restoration project; identification of objectives, monitoring and assessment. Future restoration evaluations of lowland hay meadows in temperate Europe will need adapted reference frameworks that would be more consistent with the current regional ecological and socio-economic context. To build a better reference framework, future research should improve the representativeness of *Arrhenatherion* meadows under conservation management by increasing their number and extending sampling to meadows outside nature reserves, i.e., meadows under an agri-environment scheme and Natura 2000 sites. It will also be a great opportunity to include baseline values of multiple ecosystem services (Wortley et al., 2013).

Both passive and active restoration efforts led to convergence in species richness, species composition and functional traits towards current targets, despite different pre-restoration states. Species richness increased at the same rate through time (2.4 species per year) in restored meadows whatever the restoration technique, and meadows restored with passive and sowing methods reached species richness similar to current references. The final species richness of meadows restored by hay transfer did not reach that of current references because of lower richness in initial states. Thus, having started with a lower number of species, meadows restored by fresh hay transfer may need more time to reach current reference species richness. All techniques showed successful restoration of species composition. Also, despite current and restored meadow flora indicating a richer nutrient context than historical meadows, restoration led to changes in functional composition. Observed patterns of changes are complex and may suggest that functional composition of restored meadow is not yet stabilized after 6 years. In contrast to pre-restoration communities dominated by mow-tolerant, long-lived grass with high SLA, restored communities showed higher contributions of short-lived dicotyledonous species, indicating fast resource-acquisition strategy, and grasses with higher LDMC, indicating a more conservative resource-acquisition strategy. These results demonstrate that adapting the restoration technique depending on the initial degradation state and the direct vicinity of the meadow is relevant. Our study highlights the efficiency of soil preparation and transfer of seed-containing plant material for restoring more impaired meadow sites. This is consistent with the outcomes of several studies that have tested the effectiveness of species introduction to restore lowland hay meadows or alluvial meadows (Edwards et al., 2007; Schmiede et al., 2012; Baasch et al., 2016; Harvolk-Schöning et al., 2020). Success of passive recovery also attests to the efficiency of management extensification as a means of restoring slightly degraded meadows with target species possibly remaining in the seed bank. This is congruent with the results of previous studies that reported a positive effect on species richness after cessation of fertilization and implementation of extensive management through mowing or grazing (Pallett et al., 2016; Van Vooren et al., 2018). In slightly altered landscapes that provide seed sources, stopping disturbance alone is a good tool to proceed successfully towards valuable meadow habitats and achieve restoration goals (Ruprecht, 2006). Postponing mowing from spring to summer was shown to be effective in promoting plant and invertebrate diversity in European meadows (Humbert et al., 2012). Similarly, a twice-a-year defoliation frequency was shown to be efficient in enhancing plant and insect richness and increasing export of potassium in meadow ecosystems (Uchida and Ushimaru, 2014; Piqueray et al., 2019). Before any restoration intervention, restoration practitioners should explore the local ecological conditions in order to decide which type of restoration action is most likely to succeed (Prach et al., 2020; Goret et al., 2021). Passive restoration should be favoured in mildly impaired sites, with low environmental stress and intermediate productivity, located in more natural and well-preserved landscapes (Prach et al., 2020). Financial and practical factors must also be considered. Passive recovery naturally requires lower costs than seed transfer, while fresh hay spreading additionally imposes organizational constraints. Fresh hay has to be transferred immediately to the receptor site after cutting, as storage would compromise seed viability due to rapid fermentation, and the large volume of fresh hay to be transferred requires proximity between donor and receptor sites, but compared to threshing, green haymaking is less time-consuming, requires only standard machinery (Blakesley and Buckley, 2016) and produces a more efficient seed harvest yield (Scotton and Ševčíková, 2017). The hay layer on the receptor site can also favor seedling establishment (Loydi et al., 2013).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MD, TG, JP, and GM contributed to the conception and design of the study. MD and TG carried out the field work. MD, AF, JP, and GM designed and performed the statistical analysis. MD and GM wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by the Fund for Research Training in Industry and Agriculture (PhD Grant – FRIA, F.R.S. – FNRS) of the Walloon Region.

Acknowledgments

The authors would like to thank the team of the LIFE project “Prairies bocagères” and Natagora for making this work possible and making the data available.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1136206/full#supplementary-material

References

Al Haj Khaled, R., Duru, M., Theau, J. P., Plantureux, S., and Cruz, P. (2005). Variation in leaf traits through seasons and N-availability levels and its consequences for ranking grassland species. *J. Veg. Sci.* 16, 391–398. doi: 10.1111/j.1654-1103.2005.tb02378.x

Allison, S. K. (2017). “Ecological restoration and environmental change” in *Routledge Handbook of Ecological and Environmental Restoration*. eds. S. K. Allison and S. D. Murphy (England: Routledge), 522–236.

Baasch, A., Engst, K., Schmiede, R., May, K., and Tischew, S. (2016). Enhancing success in grassland restoration by adding regionally propagated target species. *Ecol. Eng.* 94, 583–591. doi: 10.1016/j.ecoleng.2016.06.062

Barak, R. S., Hipp, A. L., Cavender-Bares, J., Pearse, W. D., Hotchkiss, S. C., Lynch, E. A., et al. (2015). Taking the long view: integrating recorded, archeological, Paleoecological, and evolutionary data into ecological restoration. *Int. J. Plant Sci.* 177, 90–102. doi: 10.1086/683394

Becking, W. R. (1957). The Zürich-Montpellier school of phytosociology. *Bot. Rev.* 23, 411–488. doi: 10.1007/BF02872328

Blakesley, D., and Buckley, P. (2016). *Grassland Restoration and Management*. London, Pelagic Publishing Ltd.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecol. Appl.* 20, 30–59. doi: 10.1890/08-1140.1

Bruchmann, I., and Hobohm, C. (2010). Halting the loss of biodiversity: endemic vascular plants in grasslands of Europe. *Grassl. Sci. Europe* 15, 776–778.

Choi, Y. D. (2007). Restoration ecology to the future: a call for new paradigm. *Restor. Ecol.* 15, 351–353. doi: 10.1111/j.1526-100X.2007.00224.x

Choi, Y. D., Temperton, V. M., Allen, E. B., Grootjans, A. P., Halassy, M., Hobbs, R. J., et al. (2008). Ecological restoration for future sustainability in a changing environment. *Écoscience* 15, 53–64. doi: 10.2980/1195-6860(2008)15[53:ERFFSI]2.0.CO;2

Cruz, P., Duru, M., Therond, O., Theau, J. P., Ducourtieux, C., Jouany, C., et al. (2002). Une nouvelle approche pour caractériser les prairies naturelles et leur valeur d’usage. *Fourrages* 172, 335–354.

Davies, C. E., Moss, D., and Hill, M. O. (2004). *Eunis Habitat Classification Revised 2004* (p. 310). Tilburg. European Environment Agency. European Topic Centre on Nature Protection and Biodiversity.

De Cáceres, M. (2020). How to use the indicspecies package (ver. 1.7.8). 30.

De Cáceres, M., Legendre, P., and Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos* 119, 1674–1684. doi: 10.1111/j.1600-0706.2010.18334.x

DEMNA. (2019). Evaluation des états de conservation des habitats et des espèces d’intérêt communautaire en Wallonie (période 2013–2018) | Natura 2000 | La biodiversité en Wallonie. Available at: http://biodiversite.wallonie.be/fr/evaluation-des-etats-de-conservation-des-habitats-et-des-especes-d-interet-communautaire-en-wallonie-periode-2013-2018.html?IDD=6292&IDC=5803

Donath, T., Hölzel, N., and Otte, A. (2003). The impact of site conditions and seed dispersal on restoration success in alluvial meadows. *Appl. Veg. Sci.* 6, 13–22. doi: 10.1111/j.1654-109X.2003.tb00560.x

Dufrêne, M., and Delescaille, L. –M.. (2005). La typologie WalEUNIS des biotopes wallons, version 1.0. Available at: http://biodiversite.wallonie.be/fr/la-typologie-waleunis-version-1-0.html?IDD=962&IDC=811

Edwards, A. R., Mortimer, S. R., Lawson, C. S., Westbury, D. B., Harris, S. J., Woodcock, B. A., et al. (2007). Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. *Biol. Conserv.* 134, 372–382. doi: 10.1016/j.biocon.2006.08.025

Emmerson, M., Morales, M. B., Oñate, J. J., Batáry, P., Berendse, F., Liira, J., et al. (2016). “Chapter two—how agricultural intensification affects biodiversity and ecosystem services” in *Advances in Ecological Research*. eds. A. J. Dumbrell, R. L. Kordas and G. Woodward, vol. *55* (Cambridge, MA: Academic Press), 43–97.

Engst, K., Baasch, A., and Bruelheide, H. (2017). Predicting the establishment success of introduced target species in grassland restoration by functional traits. *Ecol. Evol.* 7, 7442–7453. doi: 10.1002/ece3.3268

European Environment Agency. (2019). *Natura 2000 Network Viewer*. Available at: https://natura2000.eea.europa.eu/#

European Environment Agency. (2020). State of nature in the EU – results from reporting under the nature directives 2013–2018 (publication no. 10/2020; 146. EEA Report). Available at: https://www.eea.europa.eu/publications/state-of-nature-in-the-eu-2020

Fagan, K. C., Pywell, R. F., Bullock, J. M., and Marrs, R. H. (2008). Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *J. Appl. Ecol.* 45, 1293–1303. doi: 10.1111/j.1365-2664.2008.01492.x

Goret, T., Janssens, X., and Godefroid, S. (2021). A decision-making tool for restoring lowland grasslands in Europe. *J. Nat. Conserv.* 63:126046. doi: 10.1016/j.jnc.2021.126046

Goret, T., Lighezzolo, P., Kints, O., Cavelier, D., André, A., Sevrin, D., et al. (2020). The “Bocage meadows” LIFE project. Assessment of 8 years of actions in favor of the biodiversity of the meadows of Fagne-Famenne (Belgium). LAYMAN’S REPORT LIFE+11 NAT/BE/001059 (p. 20). Natagora. Available at: https://www.lifeprairiesbocageres.eu/fileadmin/Life/Prairies_bocageres/laymans_report_FINAL_EN_basseQ.pdf

Gornish, E. S., and Tylianakis, J. M. (2013). Community shifts under climate change: mechanisms at multiple scales. *Am. J. Bot.* 100, 1422–1434. doi: 10.3732/ajb.1300046

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., and Wiezik, M. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodivers. Conserv.* 22, 2131–2138. doi: 10.1007/s10531-013-0537-x

Harris, J. A., Hobbs, R. J., Higgs, E., and Aronson, J. (2006). Ecological restoration and global climate change. *Restor. Ecol.* 14, 170–176. doi: 10.1111/j.1526-100X.2006.00136.x

Harvolk-Schöning, S., Michalska-Hejduk, D., Harnisch, M., Otte, A., and Donath, T. W. (2020). Floodplain meadow restoration revisited: long-term success of large scale application of diaspore transfer with plant material in restoration practice. *Biol. Conserv.* 241:108322. doi: 10.1016/j.biocon.2019.108322

Hautier, Y., Niklaus, P. A., and Hector, A. (2009). Competition for light causes plant biodiversity loss after eutrophication. *Science* 324, 636–638. doi: 10.1126/science.1169640

Higgs, E., Falk, D. A., Guerrini, A., Hall, M., Harris, J., Hobbs, R. J., et al. (2014). The changing role of history in restoration ecology. *Front. Ecol. Environ.* 12, 499–506. doi: 10.1890/110267

Higgs, E., and Jackson, S. T. (2017). “The role of history in restoration ecology,” in *Routledge Handbook of Ecological and Environmental Restoration*. eds. S. K. Allison and S. D. Murphy (London: Routledge), 522–236.

Hodgson, J. G., Wilson, P. J., Hunt, R., Grime, J. P., and Thompson, K. (1999). Allocating C-S-R plant functional types: a soft approach to a hard problem. *Oikos* 85, 282–294. doi: 10.2307/3546494

Holl, K. D., and Cairns, J. R. (2002). “Monitoring and appraisal” in *Handbook of Ecological Restoration. Volume 1. Principles of Restoration*. eds. M. R. Perrow and A. J. Davy (Waldén: Cambridge University Press), 409–432.

Hölzel, N., Buisson, E., and Dutoit, T. (2012). Species introduction—a major topic in vegetation restoration. *Appl. Veg. Sci.* 15, 161–165. doi: 10.1111/j.1654-109X.2012.01189.x

Hothorn, T., Bretz, F., Westfall, P., Heiberger, R. M., Schuetzenmeister, A., and Scheibe, S. (2021). *Package ‘multcomp’. Simultaneous inference in general parametric models (1.4–16) [computer software]*. Available at: https://CRAN.R-project.org/package=multcomp

Humbert, J.-Y., Pellet, J., Buri, P., and Arlettaz, R. (2012). Does delaying the first mowing date benefit biodiversity in meadowland? *Environ. Evid.* 1:9. doi: 10.1186/2047-2382-1-9

Hunt, R., Hodgson, J. G., Thompson, K., Bungener, P., Dunnett, N., and Askew, A. (2004). A new practical tool for deriving a functional signature for herbaceous vegetation. *Appl. Veg. Sci.* 7, 163–170. doi: 10.1111/j.1654-109X.2004.tb00607.x

Isselstein, J., Jeangros, B., and Pavlu, V. (2005). Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe—a review. *Agron. Res.* 3, 139–151.

Jackson, S. T., and Hobbs, R. J. (2009). Ecological restoration in the light of ecological history. *Science* 325, 567–569. doi: 10.1126/science.1172977

Jones, H. P., Jones, P. C., Barbier, E. B., Blackburn, R. C., Rey Benayas, J. M., Holl, K. D., et al. (2018). Restoration and repair of Earth’s damaged ecosystems. *Proc. R. Soc. B* 285:20172577. doi: 10.1098/rspb.2017.2577

Julve, P. (1998). *Baseflor. Index botanique, écologique et chorologique de la flore de France. Version: 22/08/2017*. Available at: Http://philippe.julve.pagesperso-orange.fr/catminat.htm

Kahmen, S., and Poschlod, P. (2004). Plant functional trait responses to grassland succession over 25 years. *J. Veg. Sci.* 15, 21–32. doi: 10.1111/j.1654-1103.2004.tb02233.x

Kassambara, A. (2021). *Package ‘rstatix’. Pipe-Friendly framework for basic statistical tests (0.7.0) [computer software]*. Available at: https://CRAN.R-project.org/package=rstatix

Kiss, R., Valkó, O., Tóthmérész, B., and Török, P. (2016). Seed bank research in central-European grasslands—an overview. In J. Murphy (Ed.), *Seed Banks: Types, Roles and Research*, J. Murphy, ed., pp. 1–34). Nova Science Publisher, Hauppauge.

Kleyer, M., Bekker, R. M., Knevel, I. C., Bakker, J. P., Thompson, K., Sonnenschein, M., et al. (2008). The LEDA Traitbase: a database of life-history traits of the northwest European flora. *J. Ecol.* 96, 1266–1274. doi: 10.1111/j.1365-2745.2008.01430.x

Kühn, I., and Klotz, S. (2002). “Systematik, Taxonomie und Nomenklatur,” in *BIOLFLOR – Eine Datenbank mit biologisch-ökologischen Merkmalen zur Flora von Deutschland (Schriftenreihe für Vegetationskunde)*. eds. S. Klotz, I. Kühn and W. Durka Available at: http://www.ufz.de/biolflor/taxonomie/taxonomie.jsp?action=filter&ID_Familie=84&ID_Gattung=225&ID_Taxonomie=865

Lambinon, J., and Verloove, F. (2015). *Nouvelle Flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des régions voisines* (*6e éd*) Jardin botanique Meise, Meise.

Lavorel, S., Díaz, S., Cornelissen, J. H. C., Garnier, E., Harrison, S. P., McIntyre, S., et al. (2007). “Plant functional types: are we getting any closer to the holy grail?” in *Terrestrial* *ecosystems in a changing world.* eds. J. G. Canadell, D. E. Pataki and L. F. Pitelka (Berlin: Springer), 149–164.

Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the holy grail. *Funct. Ecol.* 16, 545–556. doi: 10.1046/j.1365-2435.2002.00664.x

Lavorel, S., Grigulis, K., McIntyre, S., Williams, N. S. G., Garden, D., Dorrough, J., et al. (2008). Assessing functional diversity in the field – methodology matters! *Funct. Ecol.* 22, 134–147. doi: 10.1111/j.1365-2435.2007.01339.x

Legendre, P., and Legendre, L. (2012). *Numerical Ecology* (*3rd ed.*) Elsevier, Amsterdam.

Loydi, A., Eckstein, R. L., Otte, A., and Donath, T. W. (2013). Effects of litter on seedling establishment in natural and semi-natural grasslands: a meta-analysis. *J. Ecol.* 101, 454–464. doi: 10.1111/1365-2745.12033

Manning, P., Gossner, M. M., Bossdorf, O., Allan, E., Zhang, Y. Y., Prati, D., et al. (2015). Grassland management intensification weakens the associations among the diversities of multiple plant and animal taxa. *Ecology* 96, 1492–1501. doi: 10.1890/14-1307.1

Marini, L., Fontana, P., Scotton, M., and Klimek, S. (2008). Vascular plant and Orthoptera diversity in relation to grassland management and landscape composition in the European Alps. *J. Appl. Ecol.* 45, 361–370. doi: 10.1111/j.1365-2664.2007.01402.x

Matson, P., Lohse, K. A., and Hall, S. J. (2002). The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio* 31, 113–119. doi: 10.1579/0044-7447-31.2.113

McGinlay, J., Gowing, D. J. G., and Budds, J. (2017). The threat of abandonment in socio-ecological landscapes: farmers' motivations and perspectives on high nature value grassland conservation. *Environ. Sci. Pol.* 69, 39–49. doi: 10.1016/j.envsci.2016.12.007

McNellie, M. J., Oliver, I., Dorrough, J., Ferrier, S., Newell, G., and Gibbons, P. (2020). Reference state and benchmark concepts for better biodiversity conservation in contemporary ecosystems. *Glob. Chang. Biol.* 26, 6702–6714. doi: 10.1111/gcb.15383

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). *Package 'vegan'. Community ecology package (2.5–7) [computer software].* Available at: https://CRAN.R-project.org/package=vegan

Pallett, D. W., Pescott, O. L., and Schäfer, S. M. (2016). Changes in plant species richness and productivity in response to decreased nitrogen inputs in grassland in southern England. *Ecol. Indic.* 68, 73–81. doi: 10.1016/j.ecolind.2015.12.024

Payne, R. J., Dise, N. B., Field, C. D., Dore, A. J., Caporn, S. J., and Stevens, C. J. (2017). Nitrogen deposition and plant biodiversity: past, present, and future. *Front. Ecol. Environ.* 15, 431–436. doi: 10.1002/fee.1528

Piqueray, J., Gilliaux, V., Decruyenaere, V., Cornelis, J.-T., Uyttenbroeck, R., and Mahy, G. (2019). Management of Grassland-like Wildflower Strips Sown on nutrient-rich arable soils: the role of grass density and mowing regime. *Environ. Manag.* 63, 647–657. doi: 10.1007/s00267-019-01153-y

Prach, K., Durigan, G., Fennessy, S., Overbeck, G. E., Torezan, J. M., and Murphy, S. D. (2019). A primer on choosing goals and indicators to evaluate ecological restoration success. *Restor. Ecol.* 27, 917–923. doi: 10.1111/rec.13011

Prach, K., Šebelíková, L., Řehounková, K., and del Moral, R. (2020). Possibilities and limitations of passive restoration of heavily disturbed sites. *Landsc. Res.* 45, 247–253. doi: 10.1080/01426397.2019.1593335

Pywell, R. F., Woodcock, B., Tallowin, J. B., Mortimer, S. R., and Bullock, J. M. (2012). Restoring species-rich grassland: principles and techniques. *Asp. Appl. Biol.* 115, 11–21.

R Core Team. (2021). *R: A language and environment for statistical computing.* Available at: https://www.r-project.org/

Ruprecht, E. (2006). Successfully recovered grassland: a promising example from Romanian old-fields. *Restor. Ecol.* 14, 473–480. doi: 10.1111/j.1526-100X.2006.00155.x

Saintenoy-Simon, J., Barbier, Y., Delescaille, L.-M., Dufrêne, M., Gathoye, J.-L., and Verté, P. (2006). *Première liste des espèces rares, menacées et protégées de la Région Wallonne (Ptéridophytes et Spermatophytes). Version 1 (7/3/2006).* Available at: http://observatoire.biodiversite.wallonie.be/especes/flore/LR2010/

Schmiede, R., Otte, A., and Donath, T. W. (2012). Enhancing plant biodiversity in species-poor grassland through plant material transfer – the impact of sward disturbance. *Appl. Veg. Sci.* 15, 290–298. doi: 10.1111/j.1654-109X.2011.01168.x

Scotton, M., and Ševčíková, M. (2017). Efficiency of mechanical seed harvesting for grassland restoration. *Agric. Ecosyst. Environ.* 247, 195–204. doi: 10.1016/j.agee.2017.06.040

Sengl, P., Magnes, M., Weitenthaler, K., Wagner, V., Erdős, L., and Berg, C. (2017). Restoration of lowland meadows in Austria: a comparison of five techniques. *Basic Appl. Ecol.* 24, 19–29. doi: 10.1016/j.baae.2017.08.004

SER. (2004). *The SER International Primer on Ecological Restoration. Society for Ecological Restoration International.* Available at: www.ser.org

Shi, Z., Sherry, R., Xu, X., Hararuk, O., Souza, L., Jiang, L., et al. (2015). Evidence for long-term shift in plant community composition under decadal experimental warming. *J. Ecol.* 103, 1131–1140. doi: 10.1111/1365-2745.12449

Stevens, C. J., David, T. I., and Storkey, J. (2018). Atmospheric nitrogen deposition in terrestrial ecosystems: its impact on plant communities and consequences across trophic levels. *Funct. Ecol.* 32, 1757–1769. doi: 10.1111/1365-2435.13063

Stevens, C. J., Dise, N. B., Mountford, J. O., and Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science* 303, 1876–1879. doi: 10.1126/science.1094678

Stoate, C., Báldi, A., Beja, P., Boatman, N. D., Herzon, I., van Doorn, A., et al. (2009). Ecological impacts of early 21st century agricultural change in Europe – a review. *J. Environ. Manag.* 91, 22–46. doi: 10.1016/j.jenvman.2009.07.005

Sullivan, E., Hall, N., and Ashton, P. (2020). Restoration of upland hay meadows over an 11-year chronosequence: an evaluation of the success of green hay transfer. *Restor. Ecol.* 28, 127–137. doi: 10.1111/rec.13063

Timmermann, A., Damgaard, C., Strandberg, M. T., and Svenning, J.-C. (2015). Pervasive early 21st-century vegetation changes across Danish semi-natural ecosystems: more losers than winners and a shift towards competitive, tall-growing species. *J. Appl. Ecol.* 52, 21–30. doi: 10.1111/1365-2664.12374

Uchida, K., and Ushimaru, A. (2014). Biodiversity declines due to abandonment and intensification of agricultural lands: patterns and mechanisms. *Ecol. Monogr.* 84, 637–658. doi: 10.1890/13-2170.1

van der Maarel, E. (1979). Transformation of cover-abundance values in phytosociology and its effects on community similarity. *Vegetatio* 39, 97–114. doi: 10.1007/BF00052021

Van der Perre, R., Bythell, S., Bogaert, P., Claessens, H., Ridremon, F., Tricot, C., et al. (2015). La carte bioclimatique de Wallonie: Un nouveau découpage écologique du territoire pour le choix des essences forestières. *Forêt. Nature* 135, 47–58.

Van Vooren, L., Reubens, B., Broekx, S., Reheul, D., and Verheyen, K. (2018). Assessing the impact of grassland management extensification in temperate areas on multiple ecosystem services and biodiversity. *Agric. Ecosyst. Environ.* 267, 201–212. doi: 10.1016/j.agee.2018.08.016

Veen, P., Jefferson, R., de Smidt, J., and van der Straaten, J.. (2009). *Grasslands in Europe: Of high nature value*, BRILL. Leiden.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional! *Oikos* 116, 882–892. doi: 10.1111/j.0030-1299.2007.15559.x

Waldén, E., and Lindborg, R. (2016). Long term positive effect of grassland restoration on plant diversity—success or not? *PLoS One* 11:e0155836. doi: 10.1371/journal.pone.0155836

Walker, K. J., Stevens, P. A., Stevens, D. P., Mountford, J. O., Manchester, S. J., and Pywell, R. F. (2004). The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biol. Conserv.* 119, 1–18. doi: 10.1016/j.biocon.2003.10.020

White, P. S., and Walker, J. L. (1997). Approximating nature's variation: selecting and using reference information in restoration ecology. *Restor. Ecol.* 5, 338–349. doi: 10.1046/j.1526-100X.1997.00547.x

Wibail, L., Goffart, P., Smits, Q., Delescaille, L.-M., Couvreur, J.-M., Keulen, C., et al. (2014). *Évaluation de l'état de conservation des habitats et espèces Natura 2000 en Wallonie. Résultats du Rapportage Article 17 au titre de la Directive 92/43/CEE pour la période 2007–2012 (p. 277).* Available at: http://biodiversite.wallonie.be/servlet/Repository/docsynthese-rapp2013-finale-26-06-2014.pdf?ID=31436&saveFile=true

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., et al. (2020). *Package 'ggplot2'. Create elegant data Visualisations using the grammar of graphics (3.3.3) [computer software].* Available at: https://CRAN.R-project.org/package=ggplot2

Wilson, J. B., Peet, R. K., Dengler, J., and Pärtel, M. (2012). Plant species richness: the world records. *J. Veg. Sci.* 23, 796–802. doi: 10.1111/j.1654-1103.2012.01400.x

Wortley, L., Hero, J. M., and Howes, M. (2013). Evaluating ecological restoration success: a review of the literature. *Restor. Ecol.* 21, 537–543. doi: 10.1111/rec.12028

Zechmeister, H. G., Schmitzberger, I., Steurer, B., Peterseil, J., and Wrbka, T. (2003). The influence of land-use practices and economics on plant species richness in meadows. *Biol. Conserv.* 114, 165–177. doi: 10.1016/S0006-3207(03)00020-X

Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 09 August 2023
DOI 10.3389/fevo.2023.1152549

Persisting effects of seed bed preparation and early grazing on plant communities in grassland restoration

Aure Durbecq[1,2]*, Renaud Jaunatre[3], Elise Buisson[4], Cassandra Favale[4], Nolwenn Maudieu[4] and Armin Bischoff[4]

[1]Aix Marseille University, Avignon University, CNRS, IRD, IMBE, Marseille, France, [2]Ecological Consultancy ECO-MED, Marseille, France, [3]University Grenoble Alpes, Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), Laboratoire des écosystémes et sociétés en montagne (LESSEM), St-Martin-d'Hères, France, [4]Avignon University, Aix Marseille University, CNRS, IRD, IMBE, Avignon, France

Check for updates

OPEN ACCESS

EDITED BY
Julien Pétillon,
University of Rennes 1, France

REVIEWED BY
Reza Erfanzadeh,
Tarbiat Modares University, Iran
Csaba Tölgyesi,
University of Szeged, Hungary

*CORRESPONDENCE
Aure Durbecq
✉ aure.durbecq@gmail.com

RECEIVED 27 January 2023
ACCEPTED 17 July 2023
PUBLISHED 09 August 2023

CITATION
Durbecq A, Jaunatre R, Buisson E, Favale C, Maudieu N and Bischoff A (2023) Persisting effects of seed bed preparation and early grazing on plant communities in grassland restoration.
Front. Ecol. Evol. 11:1152549.
doi: 10.3389/fevo.2023.1152549

Semi-natural mountain grasslands are species-rich and provide a wide range of ecosystem services, such as biomass production for livestock grazing and carbon sequestration. Land abandonment but also habitat destruction are threatening these ecosystems highlighting the need for restoration when degraded. The transfer of plant material containing seed collected at non-degraded reference sites is increasingly used for restoration but the appropriate methods to prepare the transfer and to manage the grassland afterwards are still under debates. This study aims at testing the effects of seed bed preparation by harrowing and livestock grazing on the establishment of transferred targets and community trajectories towards the reference system. In a previous study, first year seedling counts showed a positive effect of seed bed preparation and a marginally significant negative effect of grazing on the seedling recruitment of the transferred species. We asked whether the results on first year seedling densities translate into significant effects on plant cover and whether these effects on plant cover change in the following three years. Both grazing and harrowing effects on species establishment were tested using a full factorial split-plot experimental design on five replicate sites. Data on species richness and vegetation cover for both the whole plant community and the transferred species were analysed during four years after seed transfer. The cover of transferred species increased whereas their species richness remained unchanged. We found a weak positive effect of harrowing on total cover whereas grazing had no effect. Seed bed preparation by harrowing had a clear positive effect on transferred target species cover. The negative effect of grazing on first-year seedling recruitment of transferred target species turned into a positive effect in the fourth year. However, this effect was only apparent in the harrowed plots resulting in a significant grazing-by-harrowing interaction. The similarity of the plant communities to the reference increased from the first to the fourth year and this increase was stronger in grazed and harrowed plots. In conclusion, the results confirmed the positive effect of seed bed preparation on restoration by seed transfer and demonstrated that initial grazing exclusion is not needed in mountain grasslands of our study area.

KEYWORDS

community ecology, exclosures, livestock grazing, near-natural restoration methods, semi-natural grassland, soil disturbance

Introduction

While grasslands occupy an important place in the maintenance and functioning of terrestrial ecosystems, their degradation is still ongoing (Bardgett et al., 2021). Since 2021-2030 was announced the decade of ecosystem restoration by the United Nations, it is becoming crucial to consider grasslands as essential ecosystems to conserve and restore terrestrial biodiversity and ecosystem services, as well as to mitigate climate change (Temperton et al., 2019; Zhao et al., 2020; Programme UNE and Nations, 2022). In their recent review, Bengtsson et al. (2019) addressed readers directly in "Grasslands are more important for ecosystem services than you might think" and detailed numerous grassland ecosystem services. Furthermore, many authors have highlighted the importance of conserving grasslands, integrating them into long-term restoration (and monitoring) projects as well as implementing initiatives to enhance their conservation (Bengtsson et al., 2019; Temperton et al., 2019; Veldman et al., 2019; Buisson et al., 2022).

To restore species-rich grasslands or, at least, to accelerate their recovery, it is widely accepted that the lack of seed dispersal must be overcome (Török et al., 2011; Halassy et al., 2016; Török et al., 2018). The seed bank often contains annual plant species or disturbance-tolerant perennials (Erfanzadeh et al., 2016; Buisson et al., 2018), but it often lacks grassland specialists (Kiss et al., 2016), in particular if the seed bank was diminished by topsoil disturbance (compaction, removal). This may be achieved by active supply of seeds, preferably locally harvested using "near-natural restoration methods" or "nature-based solutions" (Tischew and Kirmer, 2007; Auestad et al., 2016; Valkó et al., 2022). These methods include transferring mown hay (Auestad et al., 2016; Kövendi-Jakó et al., 2019) or seed containing material collected by a brush harvester (Albert et al., 2019; Durbecq et al., 2022) from nearby non-degraded grasslands (Kiehl et al., 2010; Török et al., 2011; Scotton et al., 2012; Scotton, 2019). Furthermore, seed addition experiments showed that enhancing restoration by sowing solely is not always successful. Plant recruitment and establishment are further limiting steps of grassland restoration (Turnbull et al., 2000; Pywell et al., 2002; Bissels et al., 2006; Clark et al., 2007; Buisson et al., 2021). Seed bed preparation by topsoil disturbance before hay or seed transfer is therefore recommended by several authors (Kiehl et al., 2010; Muller et al., 2014; Klaus et al., 2017; Bischoff et al., 2018). Soil disturbance by ploughing, tilling or harrowing at shallow depth (<20 cm) is suggested to enhance the recruitment of the sown species (Hölzel et al., 2003; Schmiede et al., 2012; Long et al., 2014; Czerwiński et al., 2018) by (i) opening space and limiting competition with pre-existing vegetation (Poschlod and Biewer, 2005; Pywell et al., 2006; Klaus et al., 2017; Bischoff et al., 2018), and (ii) improving environmental conditions for germination and seedling recruitment, such as the creation of micro-reliefs increasing seed-soil adhesion (Chambers, 2000; Isselin-Nondedeu et al., 2006; Kiehl et al., 2010). However, soil disturbance may reduce the protecting effect of existing vegetation limiting high solar radiation and desiccation of young seedlings (Gibson, 2009).

The creation of recruitment gaps can also be achieved *via* livestock trampling (Kladivová and Münzbergová, 2016). European semi-natural grasslands are shaped by extensive grazing or mowing management (Gibson, 2009; Kuneš et al., 2015). Traditional grazing by livestock is needed to prevent natural succession towards woody vegetation and favours the recruitment of typical grassland species (Hejcman et al., 2013). Moreover, as long-term post-restoration management measure, extensive grazing may increase the establishment of semi-natural grassland target species by creating regeneration gaps (Török et al., 2011; Freitag et al., 2021; Tölgyesi et al., 2022). However, at the beginning of ecological restoration including seed addition, the restoration site may need fencing to protect the seedlings of sown species from livestock trampling and feeding during the first years after sowing (Bakker, 2003; Rother et al., 2013; Buisson et al., 2015; Vidaller et al., 2019).

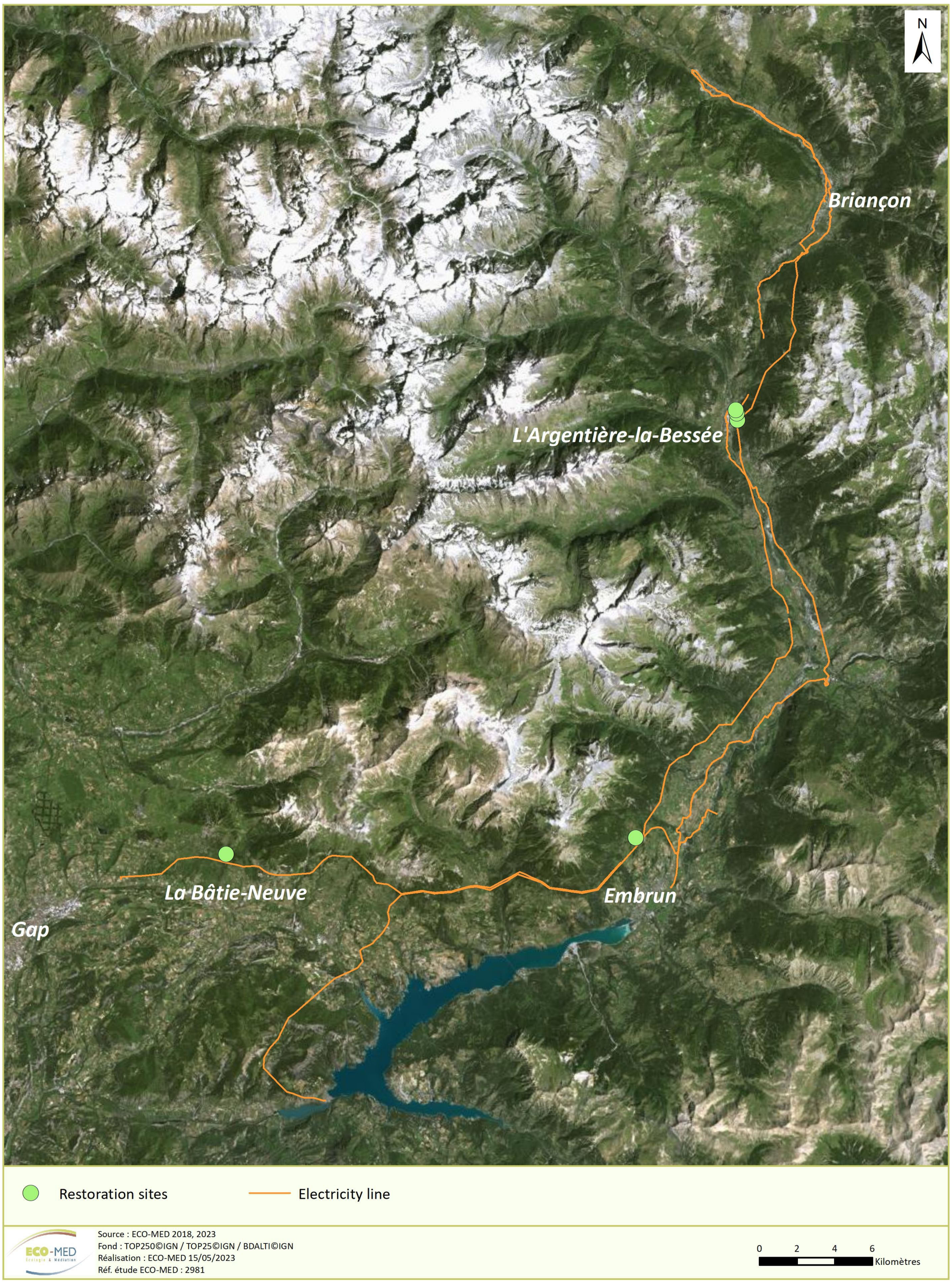
Our objective was to assess the effects of seed bed preparation by harrowing and the effect of initial grazing. The grazing exclusion treatments were fenced only the first two years after sowing while traditional grazing management was applied thereafter. First year seedling counts showed a positive effect of seed bed preparation on seedling recruitment and that grazing had a marginally significant negative effect on seedling recruitment of transferred species (Durbecq et al., 2021). In this study, we asked whether the results on first year seedling densities translate in significant effects on plant cover and whether these effects on plant cover change in the following years. More specifically, we addressed the following questions: (i) does the weak negative effect on seedling recruitment change to a positive effect in the following three years? (ii) does the positive effect of harrowing observed in the beginning persists over the following years and may thus affect the final outcome of restoration? (iii) does the mid-term grazing effect depend on seed bed preparation resulting in a significant interaction of both treatments?

We first hypothesize that an initial negative effect of grazing on transferred species changes into a positive effect after establishment since typical grassland species are favoured by grazing in the long run (Saatkamp et al., 2018; Martin et al., 2022), and that both annual weeds and ruderal plants dominating in early succession later disappear (Labadessa et al., 2020). We further hypothesize that seed preparation has a long-term positive effect on mountain grassland restoration in limiting competition by undesired perennial species and facilitating seedling recruitment of transferred species. (Freitag et al., 2021; Martin et al., 2022).

Methods

Study sites location and description

The study was conducted at five degraded mountain grassland sites in the Southern Alps, in the French Hautes-Alpes department (Figure 1). The degradation was caused by the renovation and extension of an electricity line including the construction of transitory access tracks and platforms, leading to the removal of vegetation and soil compaction-decompaction in spring 2018. The rapid return of species-rich semi-natural grasslands was identified as major objective to compensate for grassland degradation. We selected sites representative of the study zone impacted by



Source : ECO-MED 2018, 2023
Fond : TOP250©IGN / TOP25©IGN / BDALTI©IGN
Réalisation : ECO-MED 15/05/2023
Réf. étude ECO-MED : 2981

FIGURE 1
Location of the study area with the three restoration sites (in green) along the construction of a new electricity line (in orange).

construction work. Selection criteria were elevation (between 1100 and 1300 meters above sea level (m asl)), traditional management by grazing not including undergazed sites with shrub encroachment and time since the end of the construction work (< 6 months). We avoided driest and most humid sites, extreme slopes, strong soil modification (gravel addition) and particularly stony sites. Three sites were located in the municipality of L'Argentière-la-Bessée (between 44°78'77''N, 6°56'32''E and 44°78'31''N, 6°56'45''E; 1,058 to 1,132 m asl). The fourth site was in Embrun (44° 58'47''N; 6°48'31''E; 1,312 m asl) and the fifth site was in La-Bâtie-Neuve (44°58'54''N, 6°20'75''E; 1,264 m asl).

The sites were all located in the upper Durance valley at the upper montane altitudinal belt where the climate is temperate to sub-Mediterranean. The Durance valley is characterized by 740 mm of annual precipitation and average July and January temperatures of 20°C and 2°C, respectively, with an annual mean of 10.7°C

(Embrun meteorological station, 863 m asl). Due to the higher elevation compared with the meteorological station, temperatures of the study sites are 1.5 to 3°C lower. At the study sites, snow and frost occur from October to April with a usually continuous snow cover in January and February.

The grasslands of the upper Durance valley are traditionally managed and maintained open by cattle or sheep grazing, and to a lesser degree by mowing. The mountain grasslands of the area are part of a transhumance system with winter/spring grazing in the valleys and summer grazing in the alpine belt. Thus, our sites were grazed twice a year in late spring and autumn with low grazing intensity (<0.5 animal units/ha). The prevailing grazing management is transhumance with winter grazing in the bottom of the valley and summer grazing in the alpine part. The montane altitudinal belt of our study site is thus grazed twice a year for a short period in late spring and autumn. At this montane level, grasslands are characterised by Mesobromion plant communities. Accordingly, they belong to the Natura 2000 priority habitat type 'Semi-natural dry grasslands of Festuco-Brometalia and scrubland facies on calcareous substrates' (N6210, EU directive habitat 92/43/EEC; Calaciura and Spinelli, 2008). Grasslands of this habitat type are often nutrient-poor and particularly species-rich (WallisDeVries et al., 2002). The main environmental drivers of plant communities are elevation, slope, aspect and bedrock (Durbecq et al., 2020).

Seed material and field experiment

Local seeds were harvested in a traditionally managed and non-degraded semi-natural grassland of the Durance valley, in Freissinières (44°73′61″ N; 6°56′72″ E; 1,100 m asl), where the climatic and soil parameters are similar to the five study sites. Plant material was collected in July 2018 (seed production period for more than 80% of species in this region) with an integrated system combining beater brush harvester and vacuum system, all mounted on a quad to allow access to narrow and steep mountain sites (Appendix 1). We obtained 2.6 kg of dry plant material harvested in 4,000 m² source grassland. Seeds represented 60% of the harvested material and vegetative parts 40%. The average density of collected seeds was 887 seeds/g of plant material (counted in ten samples of 0.5 g of harvested plant material).

We established a full-factorial split-plot experiment at the five replicate study sites with seed bed preparation and seeding as split-plot factors, and grazing as whole-plot factor (see Durbecq et al., 2021). This resulted in eight plots of 4 m × 4 m treatments arranged in rectangles with 1 m distance between treatments (except for one site where they were separated by 50 cm distance due to lack of space; total = 40 plots). After mowing the already existing vegetation with a brush cutter, seed bed preparation and seeding were randomly assigned to eight plots within half-blocks: four plots exposed to livestock grazing comprising i) no seeding, no seed bed preparation (including removing existing vegetation), ii) seeding only, iii) seeding and seed bed preparation, iv) seed bed preparation only. The remaining four other plots, comprising the same treatments were fenced to exclude grazing. The non-sown control plots were only used to distinguish transferred and spontaneously emerging species but not included in the statistical analysis.

In October 2018, we implemented the seed bed preparation treatment by harrowing the soil (8 cm depth) using a rotary cultivator (Appendix 1). For the seeding treatment, we adjusted sown seed density by weight using 3.75 g/m² of seeds which corresponds to 104 g of harvested plant material per plot and approximately 5,700 seeds/m². This density is at the upper range of seed densities recommended for alpine grassland restoration projects in the Southern French Alps (2-4 g/m², Koch et al., 2015). The first half-blocks were fenced before the grazing periods and the other half-blocks were exposed to the local extensive grazing twice a year, in October and June. The exclosures were removed after the second-year vegetation survey, in September 2020. The three sites located in l'Argentière-la-Bessée were grazed by sheep and the two other sites by cattle corresponding to the sheep and cattle grazing ratio of the study region. The sites were neither irrigated nor fertilised.

Measurements and data analysis

Vegetation surveys were conducted at the restoration sites and at the source site of plant material transfer. The cover of all vascular plant species was visually estimated as the vertical projection of all above-ground plant organs.

For each plant species of the source site, percentage cover was estimated in five quadrats of 3 m × 3 m in early June 2018. The total plant cover for each quadrat may exceed 100% due to overlapping vegetation (Londo, 1976). Additionally, the proportion of individuals with mature seeds was estimated for all species occurring at the source site just before the harvest in July 2018. We found altogether 58 species with seeds (Appendix 2).

At restored sites, the 3 m × 3 m quadrats were located in the centre of the 4 m × 4 m quadrats to avoid edge effects, in June 2019, 2020, 2021 and 2022. For each site, species occurring in each sown treatment were compared to control plots, and those occurring in the control were identified as spontaneously emerging species. Species with ripe seeds occurring at the source site of plant material transfer were considered as "harvested species" (58 species, Appendix 2). Those harvested species that did not occur in the four control plots of the same restoration site (with largely higher cover in the seed transfer plots, ratio > 5:1) were identified as "transferred species" (27 species, Table 1). We can, however, not exclude that some species with seeds were finally not harvested because seeds or fruits were too low in the canopy or still strongly attached to the plants. Furthermore, the method may underestimate the density of transferred species since the occurrence of a species in unsown control plots does not necessarily mean that the species was not transferred.

Analysis

We used cover and species richness of the whole plant community (including both transferred species and species

TABLE 1 List of transferred species.

Alyssum alyssoides	*Festuca rubra*	*Onobrychis viciifolia*
Arrhenatherum elatius	*Festuca valesiaca*	*Pimpinella saxifraga*
Astragalus danicus	*Galium glaucum*	*Plantago media*
Bromopsis erecta	*Galium verum*	*Picris hieracioides*
Bunium bulbocastanum	*Heracleum sphondylium*	*Ranunculus bulbosus*
Camelina microcarpa	*Hypericum perforatum*	*Rhinanthus alectorolophus*
Campanula glomerata	*Knautia arvensis*	*Salvia pratensis*
Centaurea jacea	*Lathyrus pratensis*	*Scorzonera hispanica*
Dactylis glomerata	*Leucanthenum vulgare*	*Silene nutans*

emerging from the soil seed bank or seed rain) and of transferred species only for each of the four years after sowing (2019, 2020, 2021 and 2022) to analyse the effect of early grazing and of seed bed preparation on grassland restoration. To focus our analyses on the effects of seed bed preparation and early grazing on both whole community and transferred species, analyses were performed with the two treatments "Grazing" and "Harrowing" and their interactions. Plant cover complied with the assumption of linear models (normality, homoscedasticity). Thus, linear mixed-effects models (LMM) were fitted using lmer function (R package lme4, Bates et al., 2015). Species richness was analysed using generalised linear mixed-effects models (GLMM) with Poisson error distribution and log-link function. No overdispersion occurred in Poisson models. In LMM and GLMM, the significance of treatments and interactions was tested using the Anova function (R package car, Fox & Weisberg 2019). The two treatments "Grazing" and "Harrowing" were fitted as fixed effects, while "site" was fitted as random effect. To consider the split plot experimental design ("Harrowing" and "Seeding" as split plot factors and "Grazing" as whole plot factor), grazing was tested against the site x grazing interaction whereas harrowing and the harrowing × grazing interactions were tested against the model error. As we were interested in the specific effect of treatments on vegetation at different years, data analyses were performed separately for each year. *Post-hoc* Tukey tests were calculated in the case of significant treatment effects or interactions to analyse which differences between treatment combinations were significant (emmeans package, Lenth, 2020).

To compare the composition of the plant communities in the different treatments in 2019 and in 2022, we applied a Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis distance (Borcard et al., 2011), and we used a permutation multivariate analysis of variance (PERMANOVA, Anderson, 2001) with 9,999 permutations to analyse whether the plant composition was significantly different between treatments (R package vegan, Oksanen et al., 2022). Similarity to reference communities was calculated as 1 - the minimum Bray-Curtis distance (Bray and Curtis, 1957) between restoration sites and the reference site. We used the minimum Bray-Curtis distance instead of the average value in order to reduce the effect of the heterogeneity of references (Durbecq et al., 2020). We analysed grazing, harrowing effects and their interaction using the same model design as in the univariate analysis. We performed all analyses using R 4.2.0 (R Core Team, 2022).

Results

Whole community

The plant cover of the whole community (considering all species) in the four sown treatments increased over years with an average total cover of 28% (all treatments combined) in 2019, 54% in 2020, 61% in 2021, and 67% in 2022 (Figure 2). The grazing treatment had no effect on total cover. In the first year after sowing (2019, Figure 2A), the harrowing × grazing interaction was significant, with a greater cover in the non-grazed and non-harrowed treatment (Table 2A). One year later (2020, Figure 2B), this interaction was marginally significant but for different reasons than in 2019: the total plant cover was lower in the grazed and non-harrowed plots leading also to a significant harrowing main effect with plant cover being lower in non-harrowed plots (Table 2A, Figure 2B). In 2021 (Table 2A, Figure 2C), the third year after sowing, there was no effect of the treatments, whereas in 2022 the total plant cover was again lower in the non-harrowed plots (marginally significant, Table 2A, Figure 2D).

The average species richness of the plant communities was 27 species in 2019 compared to an average of 32 species for the following three years (Figure 3). No difference in total species richness was observed between the treatments and the interaction was never significant (Table 2B).

The NMDS ordination revealed a clear separation of plant community composition between restoration treatments in 2019 and in 2022 (Figure 4). 2022 polygons were smaller than 2019 polygons and approached the source site in terms of species composition, indicating higher species similarity with the source site after three years. However, the source site was still clearly separated from the restoration sites in 2022. The PERMANOVA showed differences in plant species composition of the plant communities between treatments, both in 2019 and 2022. In 2019, a marginally significant effect of the grazing treatment was found ($F_{1,19} = 1.50^{(*)}$), with typical species of the grazed plots occurring more on the bottom of NMDS axis 2, such as *Alyssum alyssoides* (L.) L., *Reseda phyteuma* L., *Eryngium campestre* L., *Centaurea paniculata* L., *Stachys recta* L., *Saponaria ocymoides* L. and *Ononis pusilla* L. Neither the harrowing effect nor harrowing × grazing interactions were significant. In 2022, the effect of grazing and the harrowing × grazing interaction on species composition were marginally significant (respectively $F_{1,19} = 1.61^{(*)}$ and $F_{1,19} = 1.71^{(*)}$), distinguishing the two non-grazed plots from the two grazed plots. The similarity to references was not significantly affected by grazing or by harrowing treatments in 2019 (χ^2_G = 0.30 and χ^2_H = 1.95). However, in 2022, grazing, harrowing and their interactions had a significant effect on similarity to the reference site ($\chi^2_G = 16.3^{***}$, $\chi^2_H = 6.40^{*}$ and $\chi^2_{G\times H} = 7.41^{**}$).

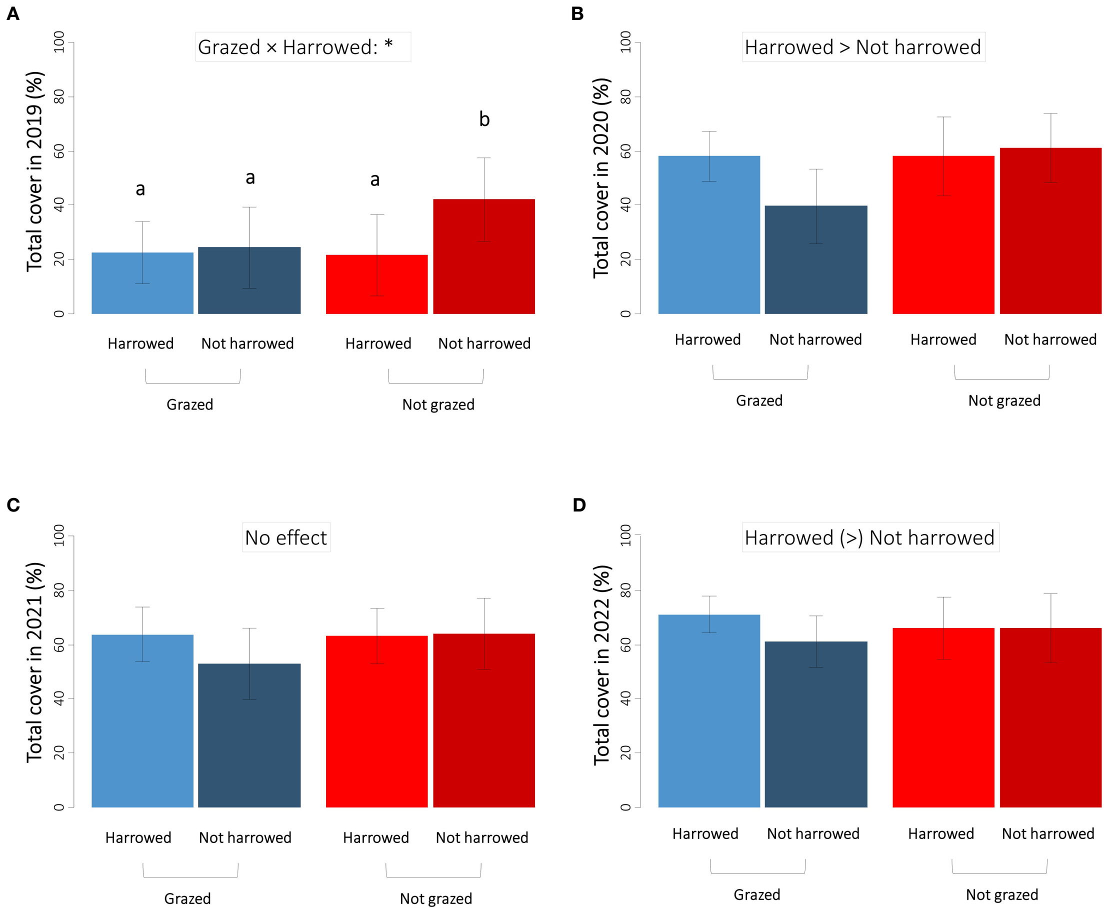


FIGURE 2
Total plant cover of the four sown treatments in **(A)** 2019, **(B)** 2020, **(C)** 2021 and **(D)** 2022. The direction of significant effects is specified using ">": $p < 0.05$ and "(>)": $p < 0.1$ (marginally significant). Significant harrowing × grazing interactions are indicated using "*" ($p < 0.05$). Error bars represent ±SE and different letters indicate significant differences between treatments ($p < 0.05$).

The grazed and harrowed plots had the highest (0.45+-0.04 SE) while the non-grazed plots had the lowest similarity to the reference site (0.31+-0.02 SE and 0.36+-0.03 SE for harrowed and not harrowed respectively, Appendix 3).

Transferred species

Altogether 27 species of the source grassland were found in the sown restoration treatments (Table 1). After correction for species

TABLE 2 Effect of grazing, harrowing and their interactions on a) cover and b) richness of the whole plant community in 2019, 2020, 2021 and 2022.

a)	Transferred sp. cover 2019			Transferred sp. cover 2020			Transferred sp. cover 2021			Transferred sp. cover 2022		
	df	χ^2	p	df	χ^2	p	df	χ^2	p	df	χ^2	p
Grazing (G)	1	0.843	0.359	1	0.318	0.573	1	2.20	0.138	1	8.68	0.003 **
Harrowing (H)	1	0.843	0.359	1	5.21	0.022 *	1	2.57	1.109	1	10.44	0.001 **
G × H	1	0.388	0.533	1	1.93	0.16	1	1.06	0.303	1	9.67	0.002 **
b)	Transferred sp. richness 2019			Transferred sp. richness 2020			Transferred sp. richness 2021			Transferred sp. richness 2022		
	df	χ^2	p	df	χ^2	p	df	χ^2	p	df	χ^2	p
Grazing (G)	1	1.15	0.284	1	0.013	0.908	1	0.937	0.333	1	1.67	0.196
Harrowing (H)	1	0.202	0.653	1	0.530	0.467	1	1.50	0.220	1	0.245	0.620
G × H	1	0.119	0.730	1	0.073	0.787	1	1.49	0.223	1	1.23	0.267

Results of LM with $\chi 2$ and significance levels: °: $p < 0.1$; *: $p < 0.05$.

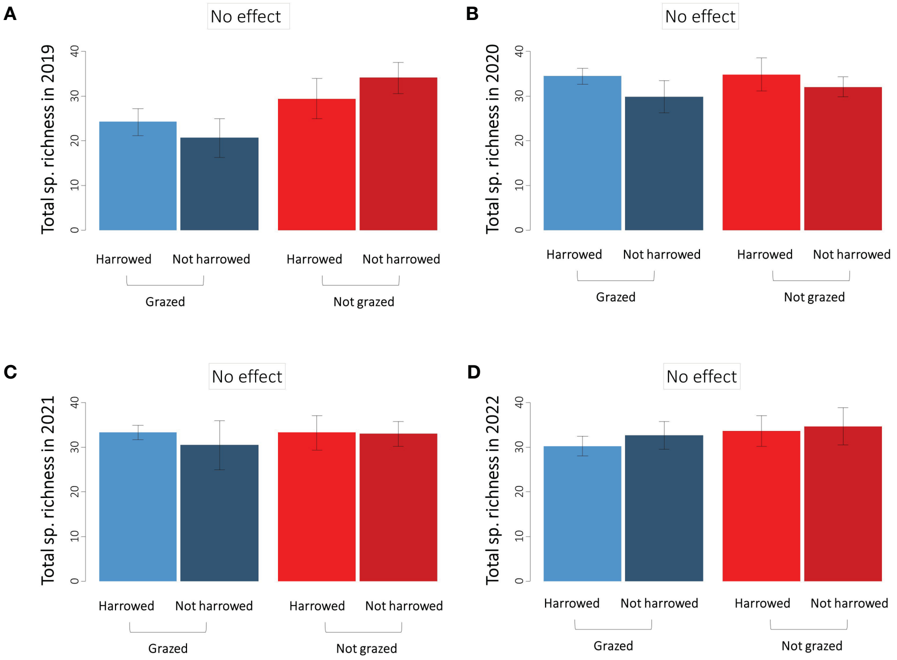


FIGURE 3
Species richness of the whole plant community in the four sown treatments in **(A)** 2019, **(B)** 2020, **(C)** 2021 and **(D)** 2022. Error bars represent ±SE.

also occurring in control treatments, the number of considered transferred species was between four and eight per plot depending on year and treatment. The cover sum of these transferred species (pooled for treatments) clearly increased over the years with, on average, 1% in 2019, 9% in 2020, 18% in 2021, and 22% in 2022 (Figure 5). In the first and third years after sowing (2019 and 2021), the treatments had no effect on the transferred species cover (Table 3A, Figures 5A, C). However, both in 2020 and 2022, transferred species cover was significantly higher in the harrowed plots, particularly when the latter where grazed (Table 3A, Figures 5B, D). The year 2022 was characterised by significant effects of both treatments and their interaction (Table 3A). Transferred species cover was the highest in the grazed and harrowed plots, averaging at 34% (Figure 5D) and representing almost half of the whole community cover (76%, Figure 2D). In grazed and non-harrowed plots, however, the cover was only 14%, and it was 24% in the non-grazed and non-harrowed plots (Figure 5D). The significant harrowing × grazing interaction

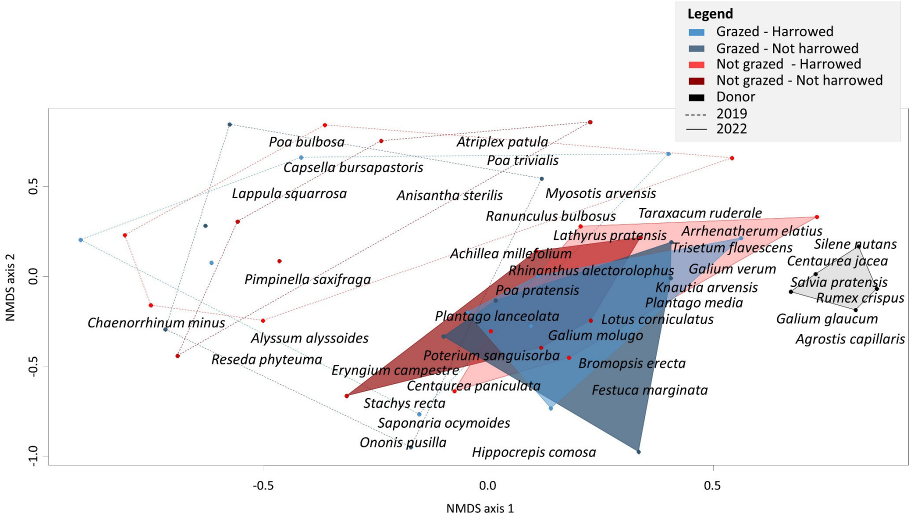


FIGURE 4
NMDS of plant species composition of the whole community in the four sown treatments in 2019 (dotted lines) and 2022 (full lines) compared to the plant species composition at the donor site. The two intermediate years 2020 and 2021 are not shown for clarity. Polygons indicate the position of the outmost plots in each treatment (stress = 0. 24). Transferred species are shown in bold.

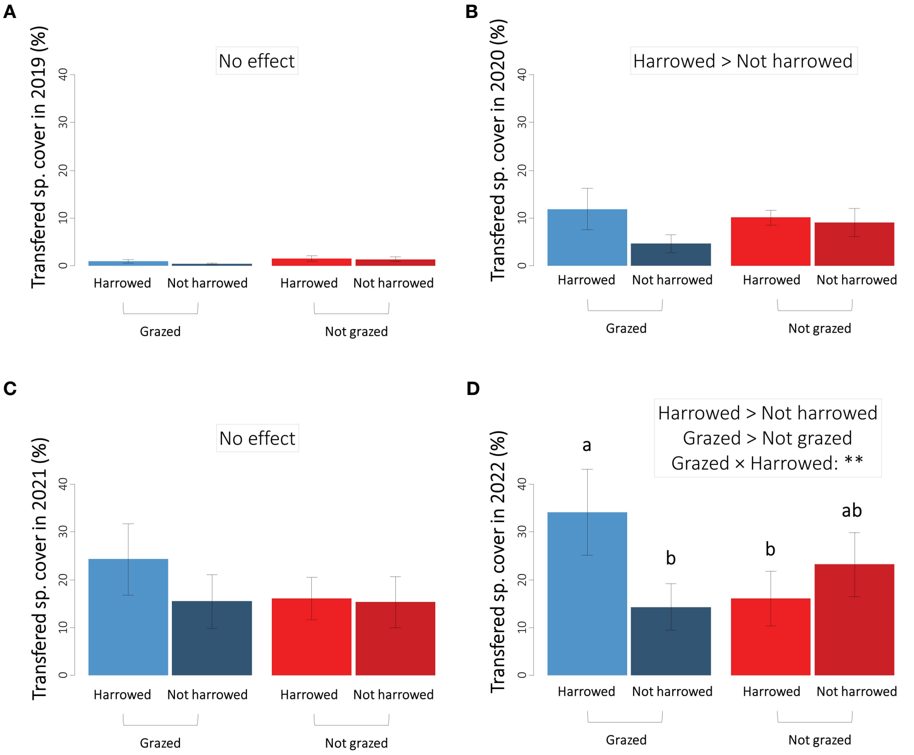


FIGURE 5
Sum of cover of transferred species in the four sown treatments in **(A)** 2019, **(B)** 2020, **(C)** 2021 and **(D)** 2022. The direction of significant effects is specified using ">": p < 0.05. Significant harrowing × (p < 0.05).

showed that the grazing effect depended on the seed bed preparation by harrowing and vice versa.

The transferred species richness was similar between treatments in the first three years after sowing (Table 3B, Figures 6A–C). We found a marginally significant effect of the grazing treatment in 2022 with slightly more transferred species in the grazed than in non-grazed plots (Table 3B, Figure 6D).

Discussion

Four years after the transfer of seed-containing plant material to degraded mountain grassland sites in the southern French Alps, transferred species reached cover values of 15 to 35% depending on the restoration treatments (Figure 5D). Seed bed preparation favoured the establishment of these species (35% in the grazed treatment). This positive effect of such soil disturbance had already been found in the first-year seedling counts (Durbecq et al., 2021) but not in first and second-year plant covers. Similarly, initial grazing increased the establishment of the transferred species by the fourth year. The small negative effect of initial grazing on first-year seedling recruitment (Durbecq et al., 2021) was not significant in the longer term and turned into a small positive effect at the end of the study period after the removal of fences. The effects of grazing on total cover and total species richness were not or marginally

TABLE 3 Effect of grazing, harrowing and their interaction on transferred species a) cover, and b) richness, in 2019, 2020, 2021 and 2022.

a)	Total cover 2019			Total cover 2020			Total cover 2021			Total cover 2022		
	df	χ^2	p	df	χ^2	p	df	χ^2	p	df	χ^2	p
Grazing (G)	1	0.024	0.877	1	0.000	1.00	1	0.004	0.950	1	0.223	0.636
Harrowing (H)	1	0.076	0.782	1	5.27	0.021 *	1	1.20	0.273	1	3.636	0.056 °
G × H	1	4.12	0.042 *	1	3.56	0.059 °	1	0.720	0.396	1	1.82	0.177
b)	Total richness 2019			Total richness 2020			Total richness 2021			Total richness 2022		
	df	χ^2	p	df	χ^2	p	df	χ^2	p	df	χ^2	p
Grazing (G)	1	2.36	0.124	1	0.012	0.914	1	0.000	1.00	1	0.906	0.341
Harrowing (H)	1	1.45	0.229	1	1.65	0.200	1	0.616	0.432	1	0.460	0.498
G × H	1	3.07	0.08	1	0.145	0.703	1	0.273	0.601	1	0.091	0.763

Results of LM with χ2 and significance levels: °, p < 0.1; *, p < 0.05.

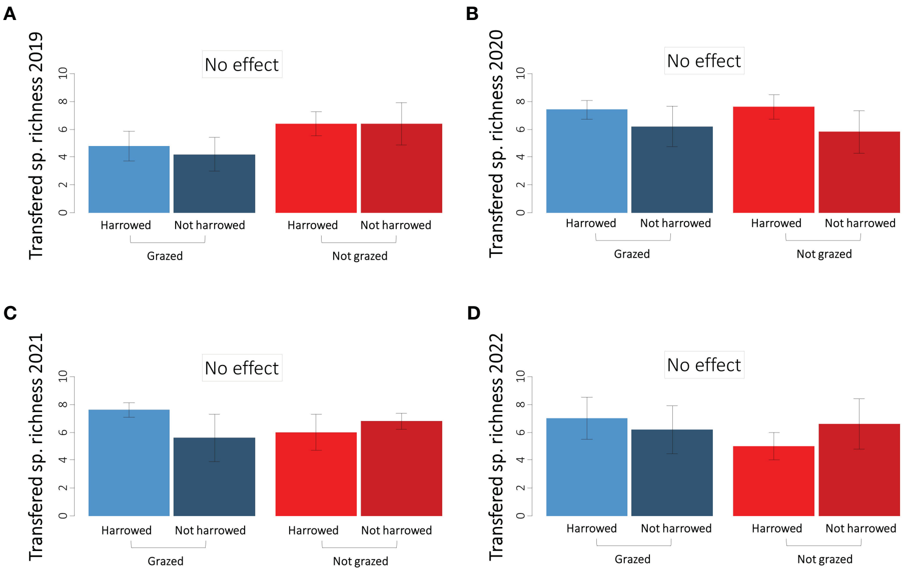


FIGURE 6
Richness of transferred species in the four sown treatments in **(A)** 2019, **(B)** 2020, **(C)** 2021 and **(D)** 2022. Error bars represent ±SE.

significant in the end showing that effects of treatments on transferred species were not strong enough to change these whole community parameters. Plant community composition, however, showed a clear development towards the reference community used as a source for seed material. This development was favoured by initial grazing whereas the effect of seed bed preparation was not significant.

The positive effects of harrowing that were observed on transferred species establishment in our restored sites indicated that the potential negative effects of seed bed preparation, such as the elimination of the protective effect of pre-existing vegetation on seedlings (e.g. against UV and drought, Gibson, 2009) were compensated by positive effects. Such positive effects include the destruction of the dense and deep root systems of competitive perennial species not targeted for restoration, reducing interspecific competition (Pywell et al., 2006; Klaus et al., 2017; Bischoff et al., 2018). Recruitment success may also be improved by a better seed adhesion to the soil after seed bed preparation (Chambers, 2000; Isselin-Nondedeu et al., 2006). Moreover, harrowing allows soil aeration increasing nitrogen mineralization (Davies et al., 2001; Steinmann, 2002). Thus, preparing the soil before seed transfer releases nutrients that increase biomass production as a short-term effect of soil tillage that disappears later on (Kristensen et al., 2003; Seabloom et al., 2003). Kiss et al. (2021) also found a beneficial effect of soil disturbance on the cover of transferred species during dry grasslands restoration after five years of revegetation monitoring. Similarly, Schmiede et al. (2012) showed improved establishment of plant species by harrowing. In a previous study on first-year seedling recruitment in our restoration sites, the number of seedlings of spontaneously emerging species was lower in the harrowed plots and the number of seedlings of transferred species and their richness were higher (Durbecq et al., 2021). Although not apparent in first-year cover, the current study confirms the positive effect of harrowing on seedling recruitment on transferred species cover in the long run. Four years after sowing the cover values of transferred species were also higher in harrowed than in non-harrowed plots. Several studies already suggested to prepare the soil before sowing but there are few studies analysing mid- to long-term effects on restoration success. In a recent meta-analysis, Slodowicz et al. (2023) found 187 publications that studied active grassland restoration, but only 13 lasted more than three years. Seed bed preparation has often positive effects on the recruitment of sown species (Edwards et al., 2007; Schmiede et al., 2012; Bischoff et al., 2018) but contrary to our mid-term results, long-term restoration studies found that such initial soil disturbance effects are less visible in the long run (Harvolk-Schöning et al., 2020; Sommer et al., 2023).

In our study, seed bed preparation had no significant effect on species richness. Similarly, Edwards et al. (2007) observed no effect of soil tillage on the restoration of calcareous grasslands monitored over four years. After grassland degradation, a rise of species richness is typically observed in the first years of restoration due to the germination of short-lived species abundant in the seed bank (Freitag et al., 2021, Valkó et al., 2022). However, Freitag et al. (2021) found a decrease in ruderal species richness over years in favour of transferred species. This effect was not observed in our study, since the transferred species richness did not change during the four years monitoring. We thus conclude that most transferred species were already recruited in the first year and just increased in cover.

Total plant cover was not affected by grazing, but our results showed a strong interaction between grazing and seed bed preparation in the beginning. The initially higher total cover in the non-grazed, non-harrowed treatment suggested that both grazing and seed bed preparation contributed to a reduction in competition the first year. This significant interaction disappeared afterwards. In several studies, grazing exclusion failed to increase grassland plant cover in early successional stages but long-term grazing maintained relatively higher diversity or stability (Milotić et al., 2010; Song et al., 2020). In our study, the plant species composition of the whole community developed towards the reference community demonstrating the success of seed transfer four years after sowing. Interestingly, initial grazing had a marginally significant positive effect on this development towards the reference community, whereas the effect of grazing on seedling recruitment of transferred species was slightly negative in a previous study on seedling recruitment (Durbecq et al., 2021).

Although the magnitude of the effect on seedling recruitment was small, our results indicated that mountain grassland communities are finally favoured by grazing even in the beginning and that an initial damage of young seedlings does not translate into a worse establishment of transferred species. Grazing is an important driver in grassland ecosystems. It influences the composition of grassland communities by creating spatial heterogeneity and constantly producing new micro-patterns by plant removal and trampling favourable for the establishment of grassland species (Kladivová and Münzbergová, 2016, Sommer et al., 2023, Martin et al., 2022). Similarly, grazing promotes environmental heterogeneity and biodiversity by redistributing and selectively concentrating nutrients (Austrheim and Eriksson, 2001). Grazing is therefore crucial to maintain the high species richness of grasslands (Dupré and Diekmann, 2001; Pykälä, 2003; Saatkamp et al., 2018). However, it remains unclear whether its intensity, frequency and/or timing drive this effect (Adler et al., 2001).

In our study, the grazing effect was still apparent after four years although grazing was only excluded for four years. Transferred species establishment was higher in the initially grazed treatments, thus explaining the development of the plant community towards the reference. Initial negative effects of grazing due to seedling damage later turned into a positive effect because grazing damages other ruderal species more than typical mountain grassland species of the reference community, thus leading to a selective effect of grazing on species establishment over the years. However, the positive grazing effect was particularly strong in the harrowed plots resulting in a significant grazing-by-harrowing interaction effect on transferred species cover in the fourth year. Freitag et al. (2021) noted that it is still not well known whether grazing interacts with soil disturbance to shape grassland species establishment, but grazing exclusion in the first years may favour transferred species that need protection against solar radiation provided by ruderal species particularly occurring in non-grazed and/or non-harrowed plots. Conversely, the non-harrowed plots may be more attractive for grazing animals than the harrowed plots due to both a higher plant biomass and palatability. In agreement with Freitag et al. (2021), our results suggest that a better understanding of interactions between grazing and seed bed preparation in driving the long-term establishment of transferred species is needed to improve management recommendations in grassland restoration involving seed-addition.

Conclusion

Sowing seeds harvested in a species-rich reference community allowed the restoration of the plant species composition of mountain grasslands towards this targeted reference state. In agreement with Labadessa et al. (2020), we found that even early extensive grazing had a small positive effect on transferred species suggesting that initial grazing exclusion is not necessary in this grassland type of our study region. Seed bed preparation had a positive effect not only on seedling recruitment but also in later stages of plant succession and can thus be strongly recommended for restoration in montane grasslands although negative effects on soil organisms and functioning need to be considered. The strong grazing-by-harrowing interaction showed that a combination of both is the best strategy to restore this grassland type. However, research is still needed to better understand how soil disturbance interacts with grazing management in the long run.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Author contributions

The project was conceived by AB, RJ and EB. Experimental design was set up by AB, AD, RJ and EB. All the authors contribute to floristic surveys. AD, CF, NM and RJ performed data analysis with assistance of the other authors. AD wrote the first draft of the manuscript. All authors contributed and approved the final version.

Funding

Financial supports were provided by ANRT (National Agency of Research and Technology) via a grant CIFRE N°2017/0478, the environmental consultancy ECO-MED, RTE (Electricity Transmission Network) and the project “France Relance” funded by the French government and the European Union.

Acknowledgments

We thank Alexis Versino, Alice Dupré-la-Tour, Charlotte Rohmer, Emile Melloul, Léo Rocher, Lola Gogniat, Margaux d’Ambly and Moritz Hammerl for field assistance. Many thanks to Daniel Pavon for his botanist expertise and Alexandre Cluchier from ECO-MED for supporting the project. We also thank the reviewers for helpful suggestions on the manuscript. The authors declare that they have no conflict of interests.

Conflict of interest

Author AD was employed by the company Ecological consultancy ECO-MED.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1152549/full#supplementary-material

References

Adler, P., Raff, D., and Lauenroth, W. (2001). The effect of grazing on the spatial heterogeneity of vegetation. *Oecologia* 128 (4), 465–479. doi: 10.1007/s004420100737

Albert, Á.-J., Mudrák, O., Jongepierová, I., Fajmon, K., Frei, I., Ševčíková, M., et al. (2019). Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. *Agric. Ecosyst. Environ.* 272, 74–82. doi: 10.1016/j.agee.2018.11.008

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* 26, 32–46.

Auestad, I., Rydgren, K., and Austad, I. (2016). Near-natural methods promote restoration of species-rich grassland vegetation—Revisiting a road verge trial after 9 years. *Restor. Ecol.* 24 (3), 381–389. doi: 10.1111/rec.12319

Austrheim, G., and Eriksson, O. (2001). Plant species diversity and grazing in the Scandinavian mountains—Patterns and processes at different spatial scales. *Ecography* 24 (6), 683–695. doi: 10.1111/j.1600-0587.2001.tb00530.x

Bakker, E. S. (2003) *Herbivores as mediators of their environment: The impact of large and small species on vegetation dynamics [Phd, S.n.]*. Available at: https://library.wur.nl/WebQuery/wurpubs/327304.

Bardgett, R. D., Bullock, J. M., Lavorel, S., Manning, P., Schaffner, U., Ostle, N., et al. (2021). Combatting global grassland degradation. *Nat. Rev. Earth Environ.* 2 (10). doi: 10.1038/s43017-021-00207-2. Article 10.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models Usinglme4. *J. Stat. Softw.* 67 (1), 1–48. doi: 10.18637/jss.v067.i01

Bengtsson, J., Bullock, J. M., Egoh, B., Everson, C., Everson, T., O'Connor, T., et al. (2019). Grasslands—More important for ecosystem services than you might think. *Ecosphere* 10 (2), e02582. doi: 10.1002/ecs2.2582

Bischoff, A., Hoboy, S., Winter, N., and Warthemann, G. (2018). Hay and seed transfer to re-establish rare grassland species and communities: How important are date and soil preparation? *Biol. Conserv.* 221, 182–189. doi: 10.1016/j.biocon.2018.02.033

Bissels, S., Donath, T. W., Hölzel, N., and Otte, A. (2006). Effects of different mowing regimes on seedling recruitment in alluvial grasslands. *Basic Appl. Ecol.* 7 (5), 433–442. doi: 10.1016/j.baae.2005.10.002

Borcard, D., Gillet, F., and Legendre, P. (2011). *Numerical Ecology with R* (New York: Springer-Verlag) 2, 688. doi: 10.1007/978-1-4419-7976-6

Bray, J. R., and Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecol. Monogr.* 27 (4), 325–349. doi: 10.2307/1942268

Buisson, E., Archibald, S., Fidelis, A., and Suding, K. N. (2022). Ancient grasslands guide ambitious goals in grassland restoration. *Science* 377 (6606), 594–598. doi: 10.1126/science.abo4605

Buisson, E., Corcket, E., and Dutoit, T. (2015). Limiting processes for perennial plant reintroduction to restore dry grasslands. *Restor. Ecol.* 23 (6), 947–954. doi: 10.1111/rec.12255

Buisson, E., De Almeida, T., Durbecq, A., Arruda, A. J., Vidaller, C., Alignan, J.-F., et al. (2021). Key issues in Northwestern Mediterranean dry grassland restoration. *Restor. Ecol.* 29, e13258. doi: 10.1111/rec.13258

Buisson, E., Jaunatre, R., Römermann, C., Bulot, A., and Dutoit, T. (2018). Species transfer via topsoil translocation: lessons from two large Mediterranean restoration projects. *Restor. Ecol.* 26, S179–S188. doi: 10.1111/rec.12682

Calaciura, B., and Spinelli, O. (2008). *Management of Natura 2000 habitats. 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (*important orchid sites). Technical Report 2008 12/24, European Commission*. Available at: http://ec.europa.eu/environment/nature/natura2000/management/habitats/pdf/6210_Seminatural_dry_grasslands.pdf.

Chambers, J. C. (2000). Seed movements and seedling fates in disturbed sagebrush steppe ecosystems: Implications for restoration. *Ecol. Appl.* 10 (5), 1400–1413. doi: 10.1890/1051-0761(2000)010[1400:SMASFI]2.0.CO;2

Clark, C. J., Poulsen, J. R., Levey, D. J., and Osenberg, C. W. (2007). Are plant populations seed limited? A critique and meta-analysis of seed addition experiments. *Am. Nat.* 170 (1), 128–142. doi: 10.1086/518565

Czerwiński, M., Woodcock, B. A., Golińska, B., and Kotowski, W. (2018). The effect of tillage management and its interaction with site conditions and plant functional traits on plant species establishment during meadow restoration. *Ecol. Eng.* 117, 28–37. doi: 10.1016/j.ecoleng.2018.03.017

Davies, M., Smith, K., and Vinten, A. (2001). The mineralisation and fate of nitrogen following ploughing of grass and grass-clover swards. *Biol. Fertil. Soils* 33, 423–434. doi: 10.1007/s003740100348

Dupré, C., and Diekmann, M. (2001). Differences in species richness and life-history traits between grazed and abandoned grasslands in southern Sweden. *Ecography* 24 (3), 275–286. doi: 10.1111/j.1600-0587.2001.tb00200.x

Durbecq, A., d'Ambly, M., Buisson, E., Jaunatre, R., Cluchier, A., and Bischoff, A. (2021). Seedling recruitment in mountain grassland restoration: Effects of soil preparation and grazing. *Appl. Veg. Sci.* 24 (1), e12564. doi: 10.1111/avsc.12564

Durbecq, A., Jaunatre, R., Buisson, E., Cluchier, A., and Bischoff, A. (2020). Identifying reference communities in ecological restoration: The use of environmental conditions driving vegetation composition. *Restor. Ecol.* 28 (6), 1445–1453. doi: 10.1111/rec.13232

Durbecq, A., Rocher, L., Jaunatre, R., Dupré la Tour, A., Buisson, E., and Bischoff, A. (2022). Mountain grassland restoration using hay and brush material transfer combined with temporary wheat cover. *Ecol. Eng.* 174, 106447. doi: 10.1016/j.ecoleng.2021.106447

Edwards, A. R., Mortimer, S. R., Lawson, C. S., Westbury, D. B., Harris, S. J., Woodcock, B. A., et al. (2007). Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. *Biol. Conserv.* 134 (3), 372–382. doi: 10.1016/j.biocon.2006.08.025

Erfanzadeh, R., KaMali, P., Ghelichnia, H., and Pétillon, J. (2016). Effect of grazing removal on aboveground vegetation and soil seed bank composition in sub-alpine grasslands of northern Iran. *Plant Ecol. Diversity* 9 (3), 309–320. doi: 10.1080/17550874.2016.1221479

Freitag, M., Klaus, V. H., Bolliger, R., Hamer, U., Kleinebecker, T., Prati, D., et al. (2021). Restoration of plant diversity in permanent grassland by seeding: Assessing the limiting factors along land-use gradients. *J. Appl. Ecol.* 58 (8), 1681–1692. doi: 10.1111/1365-2664.13883

Fox, J., and Weisberg, S. (2019). *Using car functions in other functions. CRAN R.*

Gibson, D. J. (2009). *Grasses and Grassland Ecology* (Oxford University: Oxford University Press).

Halassy, M., Singh, A. N., Szabó, R., Szili-Kovács, T., Szitár, K., and Török, K. (2016). The application of a filter-based assembly model to develop best practices for Pannonian sand grassland restoration. *J. Appl. Ecol.* 53 (3), 765–773. doi: 10.1111/1365-2664.12618

Harvolk-Schöning, S., Michalska-Hejduk, D., Harnisch, M., Otte, A., and Donath, T. W. (2020). Floodplain meadow restoration revisited: Long-term success of large scale application of diaspore transfer with plant material in restoration practice. *Biol. Conserv.* 241, 108322. doi: 10.1016/j.biocon.2019.108322

Hejcman, M., Hejcmanová, P., Pavlů, V., and Beneš, J. (2013). Origin and history of grasslands in Central Europe – a review. *Grass Forage Sci.* 68 (3), 345–363. doi: 10.1111/gfs.12066

Hölzel, N., Otte, A., and Bakker, J. P. (2003). Restoration of a species-rich flood meadow by topsoil removal and diaspore transfer with plant material. *Appl. Veg. Sci.* 6 (2), 131–140. doi: 10.1658/1402-2001(2003)006[0131:ROASFM]2.0.CO;2

Isselin-Nondedeu, F., Rey, F., and Bédécarrats, A. (2006). Contributions of vegetation cover and cattle hoof prints towards seed runoff control on ski pistes. *Ecol. Eng.* 27 (3), 193–201. doi: 10.1016/j.ecoleng.2006.02.006

Kiehl, K., Kirmer, A., Donath, T. W., Rasran, L., and Hölzel, N. (2010). Species introduction in restoration projects – Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic Appl. Ecol.* 11 (4), 285–299. doi: 10.1016/j.baae.2009.12.004

Kiss, R., Deák, B., Tóthmérész, B., Miglécz, T., Tóth, K., Török, P., et al. (2021). Establishment gaps in species-poor grasslands: Artificial biodiversity hotspots to support the colonization of target species. *Restor. Ecol.* 29 (S1), e13135. doi: 10.1111/rec.13135

Kiss, R., Valkó, O., Tóthmérész, B., and Török, P. (2016). "Seed bank research in Central-European grasslands - An overview," in *Seed Banks: Types, Roles and Research* (Nova Science Publisher), 1–34.

Kladivová, A., and Münzbergová, Z. (2016). Interacting effects of grazing and habitat conditions on seedling recruitment and establishment. *J. Veg. Sci.* 27 (4), 834–843. doi: 10.1111/jvs.12395

Klaus, V. H., Schäfer, D., Kleinebecker, T., Fischer, M., Prati, D., and Hölzel, N. (2017). Enriching plant diversity in grasslands by large-scale experimental sward disturbance and seed addition along gradients of land-use intensity. *J. Plant Ecol.* 10 (4), 581–591. doi: 10.1093/jpe/rtw062

Koch, E. M., Spiegelberger, T., Barrel, A., Bassignana, M., Curtaz, A., Spiegelberger, T., et al. (2015). *Les semences locales dans la restauration écologique en montagne—Production et utilisation de mélanges pour la préservation* (Institut Agricole Régional), 98. Available at: https://hal.inrae.fr/hal-02607421.

Kövendi-Jakó, A., Halassy, M., Csecserits, A., Hülber, K., Szitár, K., Wrbka, T., et al. (2019). Three years of vegetation development worth 30 years of secondary succession in urban-industrial grassland restoration. *Appl. Veg. Sci.* 22 (1), 138–149. doi: 10.1111/avsc.12410

Kristensen, H. L., Debosz, K., and McCarty, G. W. (2003). Short-term effects of tillage on mineralization of nitrogen and carbon in soil. *Soil Biology and Biochemistry* 35 (7), 979–986.

Kuneš, P., Svobodová-Svitavská, H., Kolář, J., Hajnalová, M., Abraham, V., Macek, M., et al. (2015). The origin of grasslands in the temperate forest zone of east-central Europe: Long-term legacy of climate and human impact. *Quat. Sci. Rev.* 116, 15–27. doi: 10.1016/j.quascirev.2015.03.014

Labadessa, R., Deák, B., and Valkó, O. (2020). No need for grazing exclusion - Sheep grazing supports grassland recovery even from the early successional stages. *Tuexenia* 40, 429–443. doi: 10.14471/2020.40.014

Lenth, R. (2020) *R. Lenth, emmeans: estimated marginal means, aka least-squares means. R package version 1.4.5.*. Available at: https://CRAN.R-project.org/package=emmeans.

Londo, G. (1976). The decimal scale for relevés of permanent quadrates. *Vegetatio* 33, 61–64.

Long, Q., Foster, B. L., and Kindscher, K. (2014). Seed and microsite limitations mediate stochastic recruitment in a low-diversity prairie restoration. *Plant Ecol.* 215 (11), 1287–1298. doi: 10.1007/s11258-014-0387-y

Martin, G., Courtial, A., Génin, A., Ramone, H., and Dutoit, T. (2022). Why grazing and soil matter for dry grassland diversity: New insights from multigroup structural equation modelling of micro-patterns. *Front. Ecol. Evol.* 10. doi: 10.3389/fevo.2022.879060

Milotić, T., Erfanzadeh, R., Pétillon, J., Maelfait, J.-P., and Hoffmann, M. (2010). Short-term impact of grazing by sheep on vegetation dynamics in a newly created salt-marsh site. *Grass Forage Sci.* 65, 121–132. doi: 10.1111/j.1365-2494.2009.00725.x

Muller, I., Mesléard, F., and Buisson, E. (2014). Effect of topsoil removal and plant material transfer on vegetation development in created Mediterranean meso-xeric grasslands. *Appl. Veg. Sci.* 17 (2), 246–261. doi: 10.1111/avsc.12059

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., O'Hara, R. B., et al. (2022) *vegan: Community Ecology Package_. R package version 2.6-2.* Available at: https://CRAN.R-project.org/package=vegan.

Poschlod, P., and Biewer, H. (2005). Diaspore and gap availability are limiting species richness in wet meadows. *Folia Geobot.* 40 (1), 13–34. doi: 10.1007/BF02803041

Programme, U. N. E., Nations, F. and A. O. of the U. (2022) *The UN Decade on Ecosystem Restoration, (2021-2030): Flagship Initiatives*. Available at: https://wedocs.unep.org/xmlui/handle/20.500.11822/37848.

Pykälä, J. (2003). Effects of restoration with cattle grazing on plant species composition and richness of semi-natural grasslands. *Biodivers. Conserv.* 12 (11), 2211–2226. doi: 10.1023/A:1024558617080

Pywell, R. F., Bullock, J. M., Hopkins, A., Walker, K. J., Sparks, T. H., Burke, M. J. W., et al. (2002). Restoration of species-rich grassland on arable land: Assessing the limiting processes using a multi-site experiment. *J. Appl. Ecol.* 39 (2), 294–309. doi: 10.1046/j.1365-2664.2002.00718.x

Pywell, R. F., Bullock, J. M., Tallowin, J. B., Walker, K. J., Warman, E. A., and Masters, G. (2006). Enhancing diversity of species-poor grasslands: An experimental assessment of multiple constraints: Enhancing diversity of species-poor grasslands. *J. Appl. Ecol.* 44 (1), 81–94. doi: 10.1111/j.1365-2664.2006.01260.x

R Core Team (2022). *R: A language and environment for stria* (Vienna, AU: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.statisticalcomputing.

Rother, D. C., Jordano, P., Rodrigues, R. R., and Pizo, M. A. (2013). Demographic bottlenecks in tropical plant regeneration: A comparative analysis of causal influences. *Perspect. Plant Ecol. Evol. Syst.* 15 (2), 86–96. doi: 10.1016/j.ppees.2012.12.004

Saatkamp, A., Henry, F., and Dutoit, T. (2018). Vegetation and soil seed bank in a 23-year grazing exclusion chronosequence in a Mediterranean dry grassland. *Plant Biosyst.* 152 (5), 1020–1030. doi: 10.1080/11263504.2017.1407375

Seabloom, E. W., Borer, E. T., Boucher, V. L., Burton, R. S., Cottingham, K. L., Goldwasser, L., et al. (2003). Competition, seed limitation, disturbance, and reestablishment of California native annual forbs. *Ecological applications* 13 (3), 575–592.

Schmiede, R., Otte, A., and Donath, T. W. (2012). Enhancing plant biodiversity in species-poor grassland through plant material transfer – the impact of sward disturbance. *Appl. Veg. Sci.* 15 (2), 290–298. doi: 10.1111/j.1654-109X.2011.01168.x

Scotton, M. (2019). Mountain grassland restoration: Effects of sowing rate, climate and soil on plant density and cover. *Sci. Total Environ.* 651, 3090–3098. doi: 10.1016/j.scitotenv.2018.10.192

Scotton, M., Kirmer, A., and Krautzer, B. (2012). *Practical handbook for seed harvest and ecological restoration of species-rich grasslands* (Cooperativa Libraria Editrice Università di Padova). Available at: https://agris.fao.org/agris-search/search.do?recordID=US201300010888.

Slodowicz, D., Durbecq, A., Ladouceur, E., Eschen, E., Humbert, J.-Y., and Arlettaz, A. (2023). The relative effectiveness of different grassland restoration methods: a meta-analysis. *Ecol. Solut. Evid.* 4, e12221. doi: 10.1002/2688-8319.12221

Sommer, L., Klinger, Y. P., Donath, T. W., Kleinebecker, T., and Harvolk-Schöning, S. (2023). Long-term success of floodplain meadow restoration on species-poor grassland. *Front. Ecol. Evol.* 10. doi: 10.3389/fevo.2022.1061484

Song, S., Zhu, J., Zheng, T., Tang, Z., Zhang, F., Ji, C., et al. (2020). Long-term grazing exclusion reduces species diversity but increases community heterogeneity in an alpine grassland. *Front. Ecol. Evol.* 8. doi: 10.3389/fevo.2020.00066

Steinmann, H. H. (2002). Impact of harrowing on the nitrogen dynamics of plants and soil. *Soil and Tillage Research* 65(1), 53–59. doi: 10.1016/S0167-1987(01)00278-1

Temperton, V. M., Buchmann, N., Buisson, E., Durigan, G., Kazmierczak, Ł., Perring, M. P., et al. (2019). Step back from the forest and step up to the Bonn Challenge: How a broad ecological perspective can promote successful landscape restoration. *Restor. Ecol.* 27 (4), 705–719. doi: 10.1111/rec.12989

Tischew, S., and Kirmer, A. (2007). Implementation of basic studies in the ecological restoration of surface-mined land. *Restor. Ecol.* 15 (2), 321–325. doi: 10.1111/j.1526-100X.2007.00217.x

Tölgyesi, C., Vadász, C., Kun, R., Csathó, A. I., Bátori, Z., Hábenczyus, A., et al. (2022). Post-restoration grassland management overrides the effects of restoration methods in propagule-rich landscapes. *Ecol. Appl.* 32 (1), e02463. doi: 10.1002/eap.2463

Török, P., Helm, A., Kiehl, K., Buisson, E., and Valkó, O. (2018). Beyond the species pool: Modification of species dispersal, establishment, and assembly by habitat restoration. *Restor. Ecol.* 26 (S2), S65–S72. doi: 10.1111/rec.12825

Török, P., Vida, E., Deák, B., Lengyel, S., and Tóthmérész, B. (2011). Grassland restoration on former croplands in Europe: An assessment of applicability of techniques and costs. *Biodivers. Conserv.* 20 (11), 2311–2332. doi: 10.1007/s10531-011-9992-4

Turnbull, L. A., Crawley, M. J., and Rees, M. (2000). Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88 (2), 225–238. doi: 10.1034/j.1600-0706.2000.880201.x

Valkó, O., Rádai, Z., and Deák, B. (2022). Hay transfer is a nature-based and sustainable solution for restoring grassland biodiversity. *J. Environ. Manage.* 311, 114816. doi: 10.1016/j.jenvman.2022.114816

Veldman, J. W., Aleman, J. C., Alvarado, S. T., Anderson, T. M., Archibald, S., Bond, W.-J., et al. (2019). Comment on "The global tree restoration potential". *Science* 366 (6463), eaay7976. doi: 10.1126/science.aay7976

Vidaller, C., Dutoit, T., Ramone, H., and Bischoff, A. (2019). Factors limiting early establishment of the Mediterranean grassland species Brachypodium retusum at disturbed sites. *Basic Appl. Ecol.* 37, 10–19. doi: 10.1016/j.baae.2019.04.003

WallisDeVries, M. F., Poschlod, P., and Willems, J. H. (2002). Challenges for the conservation of calcareous grasslands in northwestern Europe: Integrating the requirements of flora and fauna. *Biol. Conserv.* 104 (3), 265–273. doi: 10.1016/S0006-3207(01)00191-4

Zhao, Y., Liu, Z., and Wu, J. (2020). Grassland ecosystem services: a systematic review of research advances and future directions. *Landscape Ecol.* 35, 793–814. doi: 10.1007/s10980-020-00980-3

Frontiers in Ecology and Evolution

TYPE Original Research
PUBLISHED 26 October 2023
DOI 10.3389/fevo.2023.1151913

OPEN ACCESS

EDITED BY
Thierry Dutoit,
UMR7263 Institut Méditerranéen de Biodiversité et D'écologie marine et continentale (IMBE), France

REVIEWED BY
Hong Hanh Nguyen,
Senckenberg Research Institute and Natural History Museum Frankfurt, Germany
Irene Petrosillo,
University of Salento, Italy

*CORRESPONDENCE
Clément Gros
clement.gros@institut-agro.fr
Hervé Daniel
herve.daniel@institut-agro.fr

RECEIVED 26 January 2023
ACCEPTED 04 October 2023
PUBLISHED 26 October 2023

CITATION
Gros C, Bulot A, Aviron S, Beaujouan V and Daniel H (2023) Both management practices and landscape influence plant communities in urban grasslands. *Front. Ecol. Evol.* 11:1151913.
doi: 10.3389/fevo.2023.1151913

Both management practices and landscape influence plant communities in urban grasslands

Clément Gros[1]*, Adeline Bulot[1], Stéphanie Aviron[2,3], Véronique Beaujouan[1] and Hervé Daniel[1]*

[1]Institut Agro, Ecole Supérieure des Agricultures, INRAE, BAGAP, Angers, France, [2]Institut Agro, Ecole Supérieure des Agricultures, INRAE, BAGAP, Rennes, France, [3]LTSER Zone Atelier Armorique, Rennes, France

The development of urban areas now requires the integration of biodiversity issues, and this leads to better consideration of their seminatural habitats. Among these habitats, urban grasslands subjected to mowing management practices are commonly promoted over lawns to enhance biodiversity in cities. Despite their ecological value, relatively little attention has been paid to the effects of urban grassland management regimes or the landscape contexts of these habitats in terms of biodiversity. This study aims to investigate the effects of mowing practices and the landscape context of urban grasslands on species diversity and composition and the ecological strategies of plant communities. In this study, 66 sites (mown grasslands) were selected in the Angers and Rennes conurbations of western France according to their management practices (regarding mowing) and landscape gradient (more or less urbanized). The results show that mowing practices and landscape composition did not affect the richness or diversity of plant species but significantly influenced the composition of communities. Partitioning analysis showed that landscape composition explained twice as much of the variance in plant species composition as mowing practices did. Landscape composition favors plant species according to their strategies, preferential habitats, and life spans. Furthermore, diversification of management practices limits the establishment of nonnative species and induces a wider range of functional strategies, as late mowing favors competitors and disfavors stress-tolerant species. Nevertheless, management practices need to be put into perspective in the context of urban grasslands. Thus, this research brings new perspectives to recommendations for the management of urban green spaces.

KEYWORDS

herbaceous green spaces, low intensity mowing, urbanization, plant traits, biodiversity conservation, meadows

1 Introduction

Among seminatural habitats in Europe, grasslands present important challenges for biodiversity conservation (Lindborg et al., 2008; Napoleone et al., 2021), especially due to their high species richness (Habel et al., 2013). In contrast to agricultural grasslands, urban grasslands remain understudied (Cochard et al., 2019) in spite of their contribution to urban biodiversity (McKinney, 2002; Fischer et al., 2013; Vega and Küffer, 2021; Mugnai et al., 2023). Urban grasslands also provide valuable ecosystem services such as improvement of human well-being (Wu, 2014), climate regulation, or recreational use (Zhao et al., 2020). The practical application of these services takes the form of nature-based solutions, such as the implantation of grasslands to reduce urban heat islands (Zhao et al., 2020). Urban grasslands are associated with different uses because their establishment results from various urban planning choices (e.g., parks, residential areas, land reserves, derelict areas) or constraints (e.g., flood-prone areas, roadsides) (Klaus, 2013). Urban grasslands can be defined as seminatural herbaceous green spaces located in moderately to strongly urbanized areas and managed by city stakeholders. Unlike other herbaceous green spaces in cities, such as lawns, urban grasslands are characterized by relatively low-intensity management practices that aim to limit the establishment of bushes and woody plants (Politi Bertoncini et al., 2012), maintain open herbaceous habitats for biodiversity, and promote seminatural aesthetic elements in cities. The most common type of urban grasslands are urban meadows, managed by mowing (Humbert et al., 2012; Klaus, 2013) and neither fertilized nor sprayed with pesticides. Because of the absence of productive goals, urban grasslands tend to be mowed late in the season, but management choices by stakeholders regarding the timing and frequency of mowing may vary widely, depending on their objectives.

Recent changes in urban management practices, especially the generalization of lower-intensity management, have been developed ahead of scientific knowledge and bring new questions about the effects of low-intensity management of urban herbaceous spaces on plant biodiversity (Aronson et al., 2017). Regarding mowing, beneficial effects can be expected from moderate disturbance frequency (e.g., a maximum of two cuts per year), as this is known to maintain relatively high plant species richness (Parr and Way, 1988; Bakker et al., 2002). However, most studies still compare the biodiversity of contrasting types of urban green spaces rather than focusing on urban grasslands *per se* (Sehrt et al., 2020). In other types of herbaceous habitats, the effect of mowing frequency has mainly been studied by comparing lawns, which do not harbor much biodiversity due to their intensive management (Klaus, 2013), to urban grasslands (Norton et al., 2019; Sehrt et al., 2020). Urban grasslands with reduced mowing frequency were found to harbor higher plant species richness and the occurrence of typical meadow species when compared with intensively managed lawns (Sehrt et al., 2020). To our knowledge, the impact of mowing timing on urban grasslands also remains underinvestigated. In agricultural grasslands, late mowing is known to allow plant species to produce more seeds (Smith et al., 2002; Leng et al., 2011; Chaudron et al., 2016) and to complete their life cycle (Jantunen et al., 2007), which could favor competitive strategies (Johansen et al., 2019). However, existing literature has found contradictory effects of late mowing on biodiversity. It can affect species richness either negatively if delayed from spring to fall or positively if delayed from spring to summer (Grime, 2001; Humbert et al., 2012; Chaudron et al., 2016). There is thus a gap in the body of knowledge regarding the effects of the diverse mowing regimes applied by city stakeholders, in terms of both frequency and timing, on the diversity of plant communities in urban grasslands.

Beyond the effects of local management practices, the flora of urban grasslands is also likely to be influenced by their landscape context. Urbanization (defined as the process of growth and densification of urban areas) is now considered one of the major threats to biodiversity (Foley et al., 2005; Grimm et al., 2008). Urbanization induces several mechanisms, including habitat destruction, that cause the fragmentation and spatial isolation of remnant habitat patches (Williams et al., 2009; Aronson et al., 2017). Such changes have been found to decrease the frequency and abundance of native plant species (Hooftman et al., 2021) in favor of exotic species (Thompson and Jones, 1999; Smart et al., 2005; Hahs et al., 2009). For urban grasslands, although the degree of spatial isolation of grasslands did not appear to induce changes in the composition of plant communities (Cochard et al., 2017), the high occurrence of buildings and other impervious surfaces in the urban matrix was found to increase the risk of extinction for native species (Williams et al., 2006). High degrees of urbanization in the landscape context of urban grasslands are likely to affect their flora through the deleterious impacts of pollution (chemical and light) and human preferences (e.g., the introduction of exotic species) (McDonnell and Pickett, 1990; Williams et al., 2009). This leads to the selection of perennial plant species and species adapted to human disturbances (Hill et al., 2002; Lososová et al., 2006; Godefroid and Koedam, 2007). Moreover, studies have shown that plants in urban contexts tend to be more tolerant of, or favored by, high soil fertility and alkalinity (Knapp et al., 2009; Williams et al., 2015; Tautenhahn et al., 2008; Hill et al., 2002; Pyšek, 1995; Roy et al., 1999). Thus, identification of the drivers of plant species diversity in urban grasslands requires not only consideration of the local management regimes applied by stakeholders but also of the degree of urbanization in the landscape context of grasslands.

Therefore, the goal of the present paper is to investigate the effects of management regimes (mowing) and landscape composition along a rural-urban gradient on plant communities in urban grasslands. More specifically, our objectives are (1) to investigate the effects of management regimes (mowing date and frequency), the urban matrix composition in urban grasslands' landscape context, and the relative influence of these two drivers on plant species composition and diversity in urban grasslands, and (2) to examine the effects of management regimes and landscape context characteristics on plant species richness and plant strategies.

Based on the literature, it is expected that urban grassland plant communities may vary in richness, diversity, and composition according to both management practices and landscape composition. However, management practices are expected to be more likely to affect communities by favoring species according to their ecological strategies, while landscape composition should favor species according to their life span and their preferential habitat. Furthermore, we expect to find few nonnative species (Albrecht and Haider, 2013; Cochard et al., 2019).

2 Methods

2.1 Studied areas

The study was carried out in two urban areas of western France: Angers (47°28′N, 0°33′W) which covers 667 km² and contains 302,000 inhabitants, and Rennes (48°06′N, 1°40′W), which covers 705 km² and contains 460,000 inhabitants (INSEE 2022 https://www.insee.fr) in the "Zone Atelier Armorique". Both urban areas belong to the Armorican massif and are characterized by a temperate oceanic climate (average annual rainfall in Rennes: 677 mm, Angers: 668 mm; average annual temperature in Rennes: 11.7°C, Angers: 11.9°C). In the two cities, the bedrock is acidic (mainly schist and altered granite). The two cities consist of a high-built town center surrounded by low-built suburban areas, in which seminatural areas (woodlands, rivers and lakes, grasslands, and green spaces) are present. The cities are embedded within a landscape of hedgerow networks connecting mosaics of annual crops, grasslands, woodlands, and hedges.

2.2 Selection of sampling sites

Urban grasslands were selected in the two urban areas with the aim of sampling grasslands distributed along a landscape gradient more or less urbanized and characterized by contrasting management regimes with regard to mowing practices. The procedure used to select the sampled grasslands is described in the following sections.

2.2.1 Characterization of the rural-urban gradient

Land-cover maps of the two conurbations were produced using QGIS software (different versions from 2020 to 2022, http://qgis.org), using French public spatial databases and satellite or aerial images to obtain similar information for both cities. First, we collected vector data of buildings, roads, forests, woods, hedgerows, and water land cover from the high-resolution BD TOPO 3-0® database (IGN, 2018) and vector data for agricultural land uses (crops, temporary and permanent grasslands) from the Graphical Parcel Register database (RPG, 2018; www.data.gouv.fr). To classify the unassigned surfaces, we calculated the Normalized Difference Vegetation Index (NDVI) from SPOT 6-7 satellite images and infrared aerial images: higher values were considered to indicate herbaceous surfaces and lower values to indicate bare soils or mineral surfaces.

These different maps were merged to produce the final continuous land-cover maps of the two conurbations. The proportion of buildings and road surfaces is a common proxy for urbanization (Hill et al., 2002; Géron et. al., 2021). Thus, all buildings, road surfaces, and other nonvegetated areas were grouped and considered "impervious surfaces". The map of impervious surfaces was then rasterized at 5 m × 5 m resolution. Moving window analysis was then used to characterize pixels according to the proportion of impervious surfaces in their landscape context (across a 500-m buffer area) in the two urban areas using CHLOE software (Boussard and Baudry, 2017). This led to the production of a final map (at 5 m × 5 m resolution) where the value of each pixel corresponded to the proportion of impervious surfaces in its surroundings. This map was used to characterize the landscape gradients in the two conurbations (Figure 1).

We also calculated the proportions of three other land-cover classes, i.e., crops, woody, and herbaceous surfaces, to describe the two urban areas. To characterize artificial habitats other than impervious surfaces, annual crops and temporary agricultural grasslands were grouped into "crops". Permanent grasslands and other nonagricultural surfaces (i.e., wastelands, herbaceous green spaces, and roadsides) were grouped as "herbaceous surfaces" (Cochard et al., 2019; Pithon et al., 2021). Forests, woods, and hedgerows were grouped as "woody surfaces".

2.2.2 Survey of management regimes on urban grasslands

Interviews with city green space managers of the two urban areas were performed in order to identify the management practices they use for mowed urban grasslands (grazed grasslands were excluded) and to identify potential sites for sampling. Three management practices that are commonly applied on urban grasslands were retained in this study: one late mowing a year (1LM) performed after July to achieve biodiversity goals, one early mowing a year (1EM) between May and July, and mowing twice or three times a year (23M) with the first mowing between May and July. Subsequently, 149 potential sites were identified where one of these management practices was applied.

2.2.3 Site selection

By crossing data on grassland management regimes and the map of landscape gradient in the two conurbations, 66 mowed grasslands (Figure 2) managed by cities were selected: 36 in the Angers area and 30 in the Rennes area. Selected grasslands were distributed along a gradient of impervious surfaces ranging from 2% to 83% at 500 m. In total, 24 of the selected grasslands were under a 1EM management regime, and 21 grasslands were under 1LM or 23M management regimes. Only grasslands larger than 0.5 ha with fixed management for at least 5 years were selected to avoid the effects of recent perturbations. We also excluded wet habitats to restrict the study to grasslands under mesophilic conditions.

In order to ensure independence between management practices and landscape context characteristics during site selection, a Tukey's test was performed to test for differences in the proportions of impervious, woody, herbaceous, and cropped surfaces according to mowing management regimes. No significant differences were found (Figure 3).

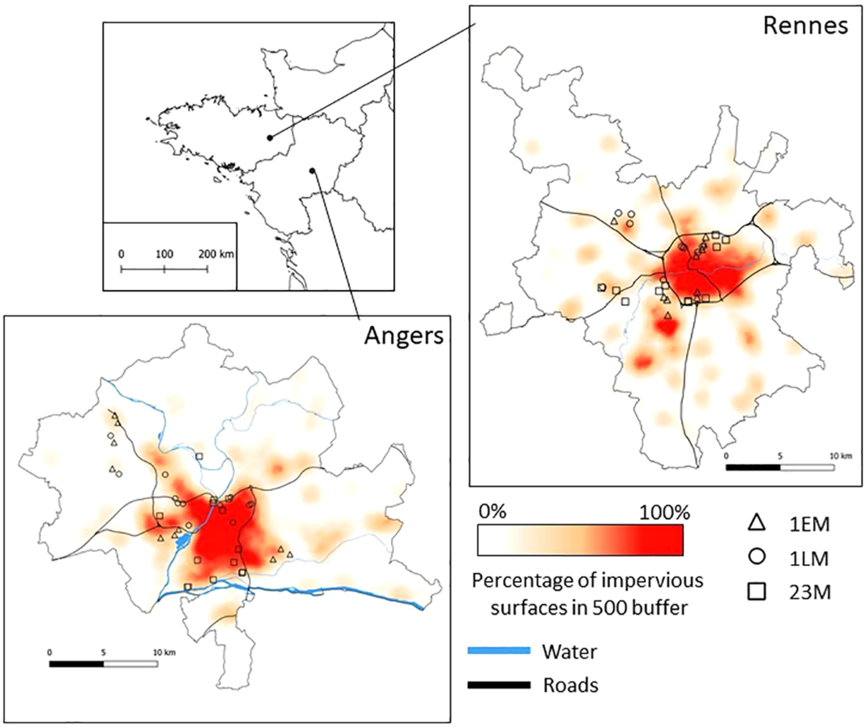


FIGURE 1
Geographical location of the two study areas and the studied grasslands with indications of management practices (mown two or three times a year (23M), mown early once a year (1EM), and mown late once a year (1LM)). Proportion of impervious surfaces was calculated in a 500-m radius around 5*5 m grids.

2.3 Vegetation survey and landscape characterization

2.3.1 Vegetation survey

In each selected grassland, five plots (330 plots in total) of 8 m^2 (2 m by 4 m) were defined with at least 5 m between each plot and from the grassland edges. In each plot, all plant species were recorded using the nomenclature defined by Tison and de Foucault (2014). An adjusted Braun-Blanquet scale method (Braun-Blanquet, 1932; Van der Maarel, 2005) was used to estimate species abundance in each plot. The fieldwork was performed in May 2021 in Angers and May 2022 in Rennes, before the first mowing of the grasslands.

Beyond the effects of mowing practices and landscape context on species richness and diversity, studying functional composition

FIGURE 2
Photo illustrating examples of the grasslands studied.

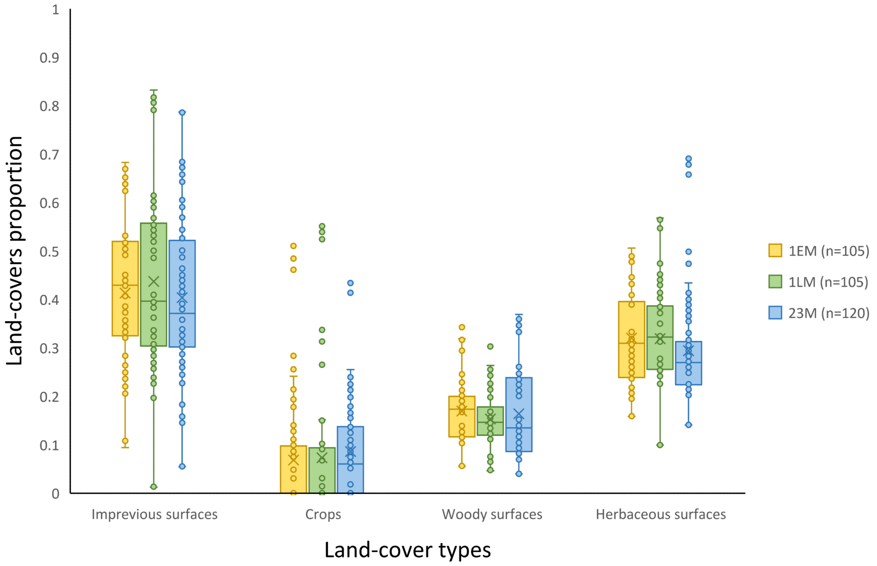


FIGURE 3
Comparison of four land-cover proportions (impervious surfaces, crops, woody surfaces, and herbaceous surfaces) within a 500-m radius of grasslands according to their management practices (mown two or three times a year (23M), mown early once a year (1EM), and mown late once a year (1LM)). Tukey's test did not show any significant differences in the proportion of each land-cover type between management practices (*p*-value > 0.05).

helps to facilitate an understanding of how plant communities respond to these variables. Thus, we collected information on plant traits and adaptive strategies. First, we collected competitor/stress-tolerator/ruderal (CSR) strategies for each species according to the database provided by Grime et al. (1988). We transformed CSR strategies into three variables that corresponded to the coordinates of each plant species in Grime's triangle: competitors (C), stress tolerators (S), and ruderals (R). A habitat preference, adapted from Julve's (2015) categories, was assigned to each species. Species characterizing meadows and grasslands (including swards or lawns) were considered grassland species, and those related to wastelands and crops were considered anthropogenic habitat species (Klotz et al., 2002). Finally, for each species, Ellenberg scores for reaction, nitrogen, and light were extracted from Julve's (2015) database.

2.3.2 Landscape characterization

Using land-cover maps of impervious surfaces and other land covers, the landscape context of each plot was described across a circular buffer with a 500-m radius according to the proportions of impervious surfaces (mean = 41%, SD = 17%, range from 2% to 83%), woody surfaces (mean = 16%, SD = 8%, range from 4% to 37%), crops (mean = 8%, SD = 11%, range from 0% to 55%), and herbaceous surfaces (mean = 31%, SD = 10%, range from 10% to 69%).

In addition, these four landscape metrics were calculated across circular buffers with radii of 250 m and 1,000 m. However, landscape metrics were highly correlated among the three radius sizes (Spearman correlation rs > 0.7; Supplementary Figure S1), suggesting high redundancy between the three spatial scales. For this reason, only the scale of 500 m was used in analyses, as it was found to optimally reflect the responses of plant communities to landscape structure (Jackson and Fahrig, 2012; Jackson and Fahrig, 2015).

2.4 Statistical analysis

2.4.1 Analyses of the composition of plant species assemblages

Canonical correspondence analysis (CCA) was performed on plant assemblages of the sampled plots to scale to test the effects of management type (one late mowing, one early mowing, and two to three mowings) and the four calculated landscape characteristics (proportions of impervious, cropped, woody, and herbaceous surfaces across a 500-m buffer area). This multivariate method is appropriate for measuring variation in plant species assemblages according to their environment (Ter Braak, 1986). To limit the strong effect of rare species, only species detected in more than 5% of plots were retained in the analyses. First, a separate CAA was undertaken to test each management and landscape variable individually. Correlations between significant variables (*p*-value < 0.05) were checked using the Pearson method to avoid multicollinearity. No variables were highly correlated (*r* < 0.7). Subsequently, variance partitioning was performed using partial CCA in order to evaluate the relative contribution of mowing practices and landscape metrics. The forward selection procedure was used to select significant mowing and landscape variables (*p*-value < 0.05) (Lepš and Šmilauer, 2003). To avoid site effects, sites were computed as block factors (a block of five replicates per grassland). All variables were significant in the global CCA. All CCAs were performed in CANOCO 5 (Ter Braak and Smilauer, 2012).

2.4.2 Analyses of species diversity, plant traits, and strategies

Generalized linear mixed models (GLMM) were used to test the effect of management type (one late mowing 1LM; one early mowing 1EM; two or three mowing 23M) and the same four landscape variables (proportions of land cover types) as in the CCA on plant species diversity (species richness and Shannon diversity index) as well as plant functional traits and strategies (CSR mean score, mean trophic level and species richness for habitat preferences and life span). The CSR score and Ellenberg index were weighed by abundance. The GLMMs were run using the lmer function of the lme4 package (Bates et al., 2015). Simple effects were tested in all models at the plot scale. Sites and urban areas were used as random effects in the models. Landscape metrics were standardized (mean = 0, SD = 1) in order to compare the effect strength (or relative importance) of the coefficients across variables. All independent variables respected the Gaussian distribution except for the proportion of crops that were log-transformed. For each plant variable, an averaged model was produced using the MuMin package (Barton, 2009); this included all models with delta AICc values lower than 15. We checked for multicollinearity in the models using the variance inflation factor (VIF) and the performance package (version 0.10.0, Lüdecke et al., 2021). The proportion of wood was excluded in the models, as this variable led to a high VIF (> 5). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality.

3 Results

3.1 Overall characteristics of plant communities in urban grasslands

A total of 153 species were observed in the 330 plots. The most common species were *Holcus lanatus* L. (85%), *Dactylis glomerata* L. (67%), *Arrhenatherum elatius* L. (65%), and *Rumex acetosa* L. (60%). Only three exotic species were found: *Oenothera* sp. (1%), *Briza maxima* L. (0.6%), and *Hordeum vulgare* L. (0.3%). The mean species richness found in 8 m² plots was 15.4 (SD = 4.13, max = 34, min = 6). The cumulative mean species richness in each grassland plot was 29 (SD = 7.05, max = 52, min = 11). The most abundant species were *Holcus lanatus* L. (frequency of 51% with a plot abundance higher than 25%), *Arrhenatherum elatius* L. (36%), *Anthoxantum odoratum* L. (18%), and *Dactylis glomerata* L. (18%).

3.2 Effects of management regimes and landscape variables on the composition of species assemblages

The arrangement of the 59 most frequent species along the two first axes of the CCA is shown in Figure 4. The forward selection procedure included the four land-cover proportions in the landscape group of the variance, partitioning CCA and mowing practices into a second group. The first two axes explained 39.5% of the relationships between plant species assemblages and environmental variables. All selected environmental variables explained 19.2% of the total variation. The first axis opposed plots surrounded by a high proportion of impervious surfaces within 500 m to plots mowed early and surrounded by high proportions of woods and herbaceous surfaces within 500 m. Plots surrounded by a high proportion of impervious surfaces were characterized by several grasses (i.e., *Avena barbata* Pott., *Trisetum flavescens* L., and *Vulpia bromoides* L.) and typical grassland dicotyledons such as *Vicia sativa* L. and *Myosotis discolor* Pers. *Daucus carota* L., while plots surrounded by a high proportion of woods and herbaceous surfaces were characterized by a wood species, *Stellaria graminea* L., and many frequently occurring grasses (*Holcus lanatus* L., *Anthoxanthum odoratum* L., *Poa pratensis* L. and *Bromus hordeaceus* L.).

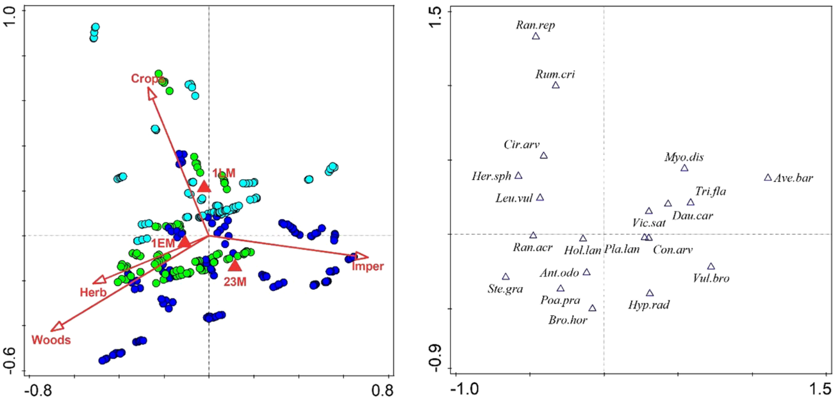


FIGURE 4
Biplot of canonical correspondence analysis representing environmental variables selected by the forward selection procedure and plot position (left) and a scatterplot of the 20 most influential species (right). For full names, see Supplementary Table S1. Arrows represent continuous landscape variables ("Proportion of Imper" for impervious surfaces, "Crops" for crop surfaces, "Wood" for woody surfaces, and "Herb" for herbaceous surfaces), and the red triangle represents mown variables (mown two or three times a year (23M), mown early once a year (1EM), and mown late once a year (1LM)) resulting from the forward selection procedure as shown. The color of the plots corresponds to mowing practices (23M plots in dark blue, 1EM plots in green, and 1LM plots in light blue).

The second axis was related to management practices and opposed plots under late mowing regimes to plots that were mowed two or three times a year. It also distinguished agricultural landscape contexts from landscapes with high proportions of herbaceous and woody surfaces, independently of the proportion of impervious surfaces (Figure 4). No grasses were associated with late mowing, but some species occurring in wastelands were found (*Cirsium arvense* (L.) Scop., *Rumex crispus* L.). Grasslands mowed only once early in the year were associated with the typical grassland species *Poaceae* (*Holcus lanatus* L., *Anthoxanthum odoratum* L., *Poa pratensis* L., and *Bromus hordeaceus* L.), while grasslands mowed two or three times a year were associated with lawn species (*Hypochaeris radicata* L. and *Plantago lanceolata* L.).

Variance partitioning showed that landscape context explained 13.2% (p-value < 0.01) of the total variance, while management practices explained 5.7% (p-value < 0.01) of the total variance (Figure 5). This represents, respectively, 68.6% and 29.7% of the explained variance. The interaction between landscape and management variables explained less than 1% of the total variance.

3.3 Effect of management type and landscape characteristics on species diversity indices and on plant strategies

GLMM did not show any significant effect of management practices or landscape composition on species richness, Shannon index, Ellenberg index, or life form species richness (Supplementary Table S2).

The GLMM performed for plant strategies showed a significant effect of management type on plant CSR scores (Table 1). Scores for competitors in late-mown grasslands (1LM) were 8% higher than in early-mown grasslands (1EM) and 11% higher than in grasslands that were mowed two or three times a year (23M) (Figure 6). Late-mown grasslands also contained fewer stress-tolerant species than early-mown grasslands and grasslands which were mowed two or three times a year (Figure 6). No significant difference was found between early mown grasslands and grasslands mown twice or three times a year for both stress-tolerant and competitor plant species (Table 1). Management type did not have significant effects on ruderal score, the species richness of grassland and anthropogenic habitats, or the species richness of annual species.

GLMM also showed a significant response of plant CSR scores to two landscape variables (Table 1). A higher proportion of impervious surfaces within a 500-m radius resulted in more ruderal plant species (Figure 7), while the proportion of crops within 500 m had a positive effect on competitor scores in plant communities and a negative effect on stress-tolerant species. The proportion of impervious surfaces also had a significant and positive effect on the species richness of annual species and of species associated with anthropogenic habitats (Table 1; Figure 8).

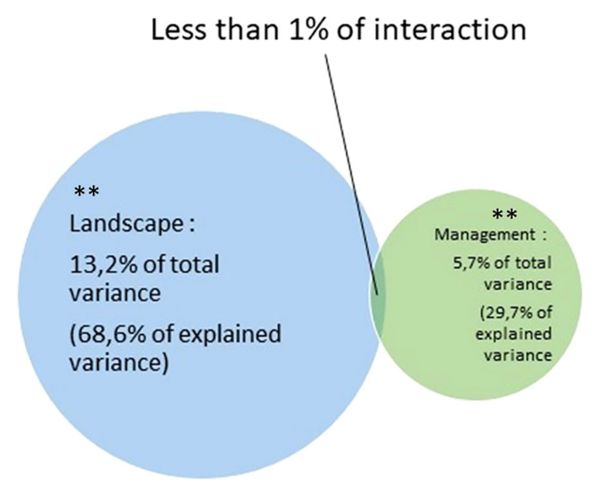


FIGURE 5
Venn diagram representation of CCA variance partitioning due to land-cover proportion and mowing practices. Both landscape and management variance explanations were significant (**: p-value < 0.01).

4 Discussion

4.1 Effect of management regimes and landscape characteristics on the diversity and composition of plant species assemblages

The total species richness (153 species) found in the 66 urban grasslands appeared to be relatively important in the studied region, especially in comparison to herbaceous green spaces with higher management intensity (i.e., mowed up to 20 times a year) in the same urban area (Chollet et al., 2018). Compared with other urban habitats, this species richness represents three times the species richness found in Paris lawns (Bertoncini et al., 2012) and is similar to species richness in the woodlands of Angers and Rennes (Vallet et al., 2008). This highlights the ecological importance of ordinary grassland habitats in urbanized areas, which previous studies have also found (Muratet et al., 2007; Threlfall et al., 2016). Furthermore, only three exotic species were observed in the present study. This result contradicts other studies that have found high occurrences of these species in urban habitats (Pyšek, 1998; Kowarik, 2008). Unlike unmanaged habitats such as wasteland, which harbors many nonnative species (Albrecht and Haider, 2013), grassland under low-intensity management practices such as mowing could maintain relatively competitive flora that may prevent the installation of nonnative species. Our study confirms the value of urban meadows for the conservation of native species in cities (English et al., 2022).

No effect of mowing practices or landscape composition was found on plant species richness and diversity in urban grasslands. We expected that lower management frequency (i.e., one cut a year compared to two or three cuts a year), as well as late mowing, would lead to higher species richness and diversity, as other studies have highlighted in the context of agricultural grasslands (Smith et al., 2000; Gaujour et al., 2012). However, our focus on a narrow range of mowing intensities (i.e., one early cut, one late cut, or two to three cuts a year) may explain the lack of response of plant richness and

TABLE 1 Final mean models for the effect of mown practices and land-cover proportion in a 500-m radius on CSR strategy score, optimal habitat, and annual species richness. Significant results, i.e. estimates whose 95% confidence interval do not include zero, are in bold.

Response variable	Independent variable	Estimate	Adj SE	*z*	Lower 95% CI	Upper 95% CI
Competitor score	Intercept	0.336	0.014	24.294	**0.309**	**0.363**
	Impervious surfaces	0.000	0.001	0.102	−0.003	0.002
	Herbaceous surfaces	0.000	0.000	0.002	0.000	0.000
	Crops	0.013	0.005	2.586	**0.003**	**0.023**
	1EM vs. 1LM	−0.027	0.013	2.146	**−0.052**	**−0.002**
	23EM vs. 1LM	−0.038	0.012	3.138	**−0.062**	**−0.014**
	23EM vs. 1EM	−0.011	0.012	0.926	−0.034	0.012
Stress tolerator score	Intercept	0.256	0.007	37.665	**0.243**	**0.269**
	Impervious surfaces	−0.004	0.005	0.751	−0.014	0.006
	Herbaceous surfaces	0.004	0.004	0.937	−0.004	0.012
	Crops	−0.009	0.004	2.126	**−0.017**	**−0.001**
	1EM vs. 1LM	0.021	0.009	2.244	**0.003**	**0.039**
	23EM vs. 1LM	0.036	0.009	3.96	**0.018**	**0.054**
	23EM vs. 1EM	0.015	0.009	1.644	−0.003	0.033
Ruderal score	Intercept	0.407	0.012	35.288	**0.384**	**0.430**
	Impervious surfaces	0.012	0.005	2.276	**0.002**	**0.022**
	Herbaceous surfaces	−0.004	0.006	0.698	−0.015	0.007
	Crops	−0.005	0.005	0.917	−0.016	0.006
	1EM vs. 1LM	0.005	0.012	0.424	−0.018	0.028
	23EM vs. 1LM	0.002	0.016	0.122	−0.030	0.034
	23EM vs. 1EM	−0.004	0.013	0.316	−0.029	0.021
Grassland habitat species richness	Intercept	9.885	0.649	15.238	**8.614**	**11.156**
	Impervious surfaces	−0.122	0.409	0.298	−0.924	0.680
	Herbaceous surfaces	−0.098	0.333	0.294	−0.751	0.555
	Crops	−0.394	0.283	1.392	−0.949	0.161
	1EM vs.1LM	0.527	0.611	0.863	−0.670	1.724
	23EM vs.1LM	0.472	0.594	0.795	−0.692	1.636
	23EM vs.1EM	−0.055	0.591	0.093	−1.214	1.104
Anthropic habitat species richness	Intercept	4.573	0.293	15.617	**3.999**	**5.147**
	Impervious surfaces	0.898	0.289	3.104	**0.331**	**1.465**
	Herbaceous surfaces	0.089	0.368	0.242	−0.632	0.810
	Crops	0.309	0.293	1.056	−0.265	0.883
	1EM vs.1LM	−0.395	0.536	0.737	−1.445	0.655
	23EM vs.1LM	−0.511	0.524	0.975	−1.538	0.516
	23EM vs.1EM	−0.116	0.520	0.223	−1.136	0.904
Annual species richness	Intercept	3.939	0.503	7.825	**2.952**	**4.926**
	Impervious surfaces	0.789	0.288	2.739	**0.224**	**1.354**
	Herbaceous surfaces	−0.206	0.378	0.545	−0.947	0.535

(Continued)

TABLE 1 Continued

Response variable	Independent variable	Estimate	Adj SE	z	Lower 95% CI	Upper 95% CI
	Crops	0.13	0.339	0.383	−0.535	0.795
	1EM vs.1LM	0.249	0.596	0.418	−0.919	1.417
	23EM vs.1LM	0.566	0.582	0.973	−0.574	1.706
	23EM vs.1EM	0.317	0.576	0.55	−0.813	1.447

diversity to grassland management. In contrast, previous studies showing the effects of mowing regimes on plant species richness have compared more contrasting intensities of management practice in urban herbaceous spaces from lawns to grasslands (Socher et al., 2012; Rudolph et al., 2017; Norton et al., 2019; Sehrt et al., 2020). Another hypothesis could be that the effects of management practices on species richness found in the literature might be related to the presence of high proportions of nonnative plants. Indeed, such species have been found by other studies to represent up to 20% of plant species richness in unmanaged habitats (Muratet et al., 2007). In the present study, the lack of species gain related to the installation of nonnative species might then explain the absence of an effect of mowing regimes on species richness and diversity.

Moreover, the lack of a significant effect of landscape composition on plant richness or diversity may be explained by the length of the landscape gradient considered in the present study. Indeed, the studied urban grasslands were distributed along a gradient ranging from moderately urbanized (2%) to highly urbanized landscapes (83%), because these extensive habitats are less frequent in rural landscapes (Fischer et al., 2013; Horák et al., 2022). Although many studies have found significant increases in plant richness in highly to moderately urbanized landscapes (McKinney, 2008), the impact of urbanization on species richness is nevertheless likely to strongly vary according to the geographical location of cities, historical and economic factors, and the spatial scale considered (McKinney, 2008). In the specific context of the present study, the landscape gradient considered may be too narrow to detect any variability in plant richness or diversity. In contrast to species richness and diversity, we found that the species composition of plant communities in urban grasslands was significantly influenced by local mowing practices and landscape composition. Analysis of plant species assemblages showed that intensively managed grasslands (i.e., mowed two to three times a year), as opposed to grasslands mowed once late in the year, were especially characterized by plant species that are typical of lawns. The strength of the influence of mowing practices on floristic composition found in the present study is comparable with that found in another study carried out in an agricultural context (Barbaro et al., 2004). This highlights the potential for the implementation of late mowing practices to add value by enhancing the composition of plant communities in urban grasslands. However, the difference in species composition between extensively and frequently mown grasslands was not associated with an increase in species richness or diversity. This suggests that there is no optimum in our range of management practices and that, on the contrary, the diversification of urban grassland management practices can lead to a wider range of floristic composition.

Plant species assemblages in urban grasslands were, more importantly, impacted by the composition of the surrounding landscapes. Grasslands with a high proportion of herbaceous and

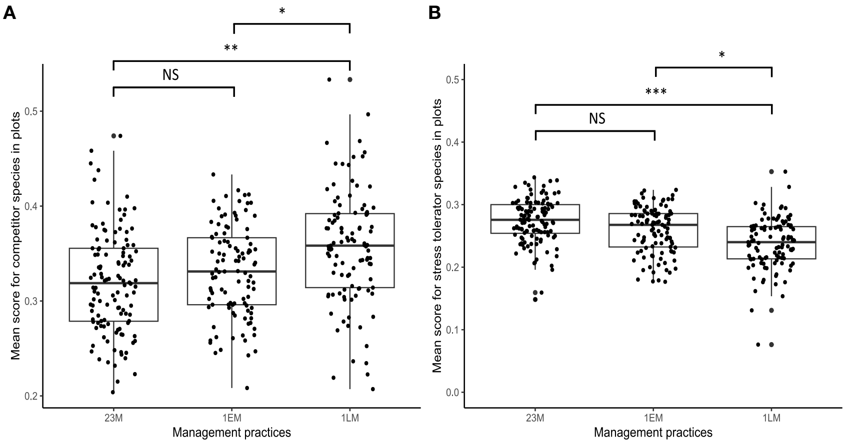


FIGURE 6
Graphical representation of the significant effect of mowing practices (mown two or three times a year (23M), mown early once a year (1EM), and mown late once a year (1LM)) on competitor score **(A)** and stress tolerator score **(B)** (n = 330) according to mean model results (*p < 0.05; **p < 0.01; ***p < 0.001).

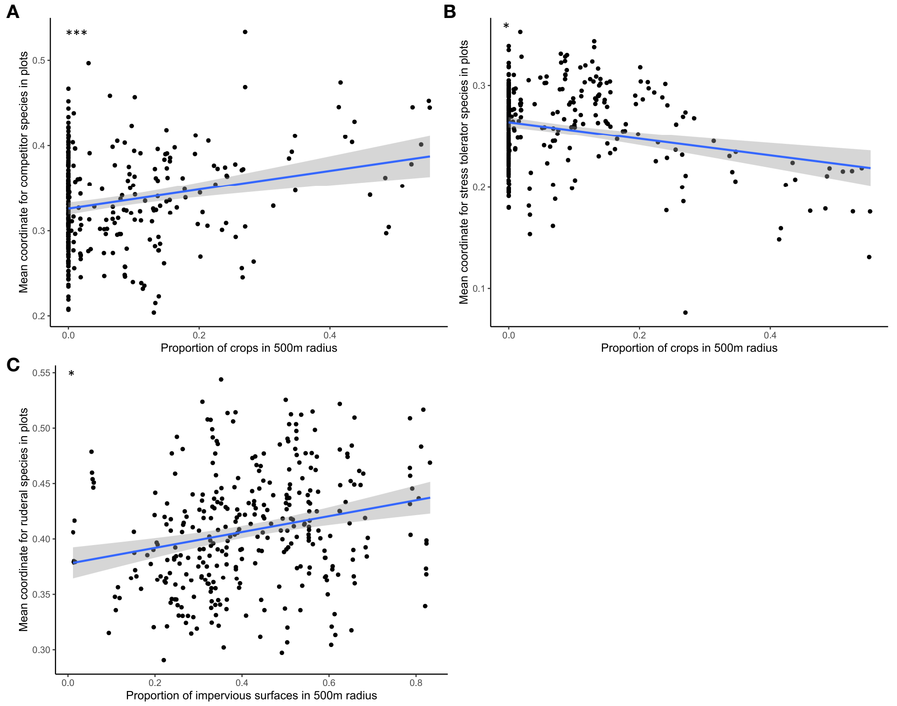


FIGURE 7
Graphical representation of significant effects of crop proportion on competitor score **(A)** and stress tolerator score **(B)** and of impervious surface proportion on ruderal score **(C)** according to mean model results (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

woody habitats in their surroundings were characterized by contrasting species assemblages in comparison with grasslands embedded in landscapes with many impervious surfaces. Along an urban-rural gradient, Cochard et al. (2019) have also observed an effect of landscape composition on the plant species assemblages of extensively managed road verges, and many grassland species seemed to be negatively affected by the increasing proportion of built-up areas in the landscape. In rural contexts, many studies have found an effect of landscape patterns on plant diversity and composition (Schmucki et al., 2012; Olsen et al., 2018; Kimberley et al., 2021). In Schmucki et al. (2012), the presence of seminatural elements in the surroundings seemed to affect the composition of plant communities and increase local plant species richness (Schmucki et al., 2012), thus contrasting with agricultural landscape contexts (Loos et al., 2021).

In urban contexts, plant communities are the result of different specific filters applied to the global species pool (Williams et al., 2009; Aronson et al., 2016), on which factors acting at both landscape and local scales induce selective pressures (Williams et al., 2015). In this study, we found an influence of both landscape composition and local management practices on species assemblages, with a greater influence of variations in landscape composition than of local mowing practices. The differences in plant community composition observed between

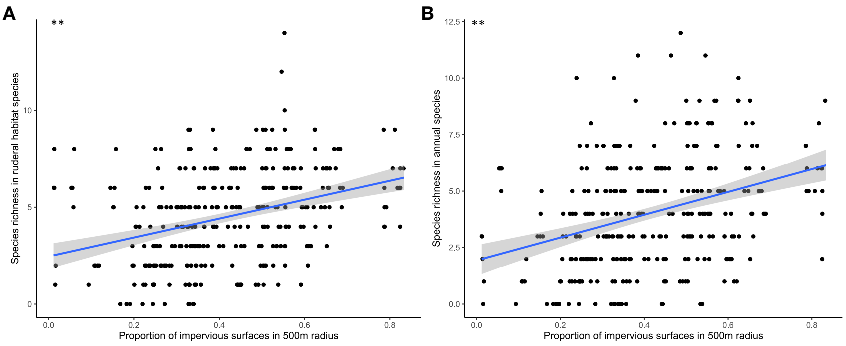


FIGURE 8
Graphical representation of impervious surface proportion on species richness of anthropogenic habitat plants **(A)** and annuals **(B)** according to mean model results (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

urban and rural grasslands can be linked to the increased effect of environmental change in the city center compared to suburban areas (i.e., water stress, pollution, and the urban heat island effect) (Williams et al., 2009). It could also be explained by the effects of landscape configuration that were not specifically considered in the present study. Habitat fragmentation (e.g., escribed via the mean proximity size index) and landscape complexity (e.g., edge density and largest patch index) can strongly affect plant composition in urban contexts (Peng et al., 2019). Habitat fragmentation was found to increase the biotic homogenization of plant communities (Zeeman et al., 2017), while landscape complexity has been shown to be positively related to plant species diversity (Peng et al., 2019). Although we did not specifically address the potential effects of landscape configuration, it has been shown in landscape ecology that landscape composition and configuration are highly correlated (Fahrig, 2003; Fahrig et al., 2011). In an urban context, higher levels of urbanization can also be associated with higher habitat fragmentation and landscape complexity (Yeh and Huang, 2009; Buyantuyev and Wu, 2010; Peng et al., 2019). Thus, the changes in plant communities found along our landscape gradients may be partially explained by variations in landscape configuration underlying landscape composition. However, distinguishing the respective effects of these two landscape components is difficult (Fahrig, 2003; Fahrig et al., 2011). Other landscape factors can act as filters for plant communities. Habitat transformation and fragmentation might also filter species, especially in relation to the dispersal strategies that determine their capacity to reach a suitable environment. Although such processes remain unclear (Williams et al., 2015), previous studies have suggested that wind-dispersed species might be more likely to disappear in urban environments (Williams et al., 2005; Sodhi et al., 2008; Knapp et al., 2010), and isolated urban grasslands may limit seed arrival (Fischer et al., 2013). Thus, further study of seed rain composition in urban contexts is needed to better understand how the landscape changes induced by urbanization drive the selection of species plant strategies in urban habitats.

4.2 Influence of mowing and landscape composition on plant strategies in urban grasslands

Although mowing practices and landscape composition did not influence plant species richness in our sample, they had a significant effect on species composition by favoring certain plant strategies. In agreement with our hypothesis, an effect of mowing practices on plant CSR strategy was found. Indeed, there were more competitive species (such as *Dactylis glomerata* L. and *Arrhenatherum elatius* L.) in late-mown than in early-mown grasslands, however frequently they were mowed (1EM or 23M). One cut before flowering could prevent plant reproduction (Gaujour et al., 2012), and a cut before seed exports could select a competitive strategy (Johansen et al., 2019). In addition, vegetation observed under late mowing conditions was denser and could prevent the establishment of new plants (Smith and Haukos, 2002), thus favoring the development of competitor species. Stress-tolerant species (such as *Lotus corniculatus* L.) were less observed in late-mown grasslands compared to early-mown grasslands (1EM and 23M). This result indicates that early management of grasslands may cause more disturbance (Kahmen et al., 2002).

Beyond the effects of local management practices on plant strategies, our results show that urban grasslands harbored less stress-tolerant and more competitive plant species when surrounded by high crop coverage in their landscape context, which contradicts the results of a previous study showing enhanced competitor strategy in more urbanized contexts (Chocholoušková and Pyšek, 2003). However, considering the complexity of urban systems and the various methods used to assess CSR strategies, the response of CSR plant traits to urbanization remained unclear (Williams et al., 2015). Actually, both types of plant strategy (i.e., stress tolerators and competitors) might reflect not only plant responses to landscape composition but also the degree of grassland disturbance (Herben et al., 2018). Indeed, a high score for ruderal species was observed in grasslands in more urbanized landscapes, as shown in Knapp et al. (2009). Since this strategy is associated with disturbed habitats, urban grasslands seem to undergo more disturbance in highly urbanized landscape contexts. In addition, the richness of annual species and of species associated with anthropogenic habitats was also higher in grasslands in more urban contexts, confirming the hypothesis that grasslands located in the most urbanized landscapes tend to be more disturbed.

5 Conclusion

Our study illustrates the importance of considering factors that act at both local and landscape scales when identifying the drivers of plant communities in urban grasslands. This study also provides evidence that assessing effects in terms of species richness is not sufficient and that community composition must also be considered. Accordingly, although mowing practices and landscape composition did not influence plant species richness or diversity, we found that they were both important drivers of the composition of plant species assemblages. Nevertheless, they affected plant communities in different ways and to different extents. Landscape composition had the strongest influence; this was mostly characterized by effects on CSR strategies, preferential habitats, and the life span of plant species. This highlights the important role of landscape composition in filtering plant species communities in urban grasslands in comparison with local drivers. Our study therefore provides evidence that favors rethinking urban grassland planning not only by changing local management practices but also by promoting the maintenance of seminatural green spaces in the landscape context. Our findings also support changes in management practices for herbaceous vegetation in cities, favoring extensive management practices such as late mowing. Such practices, which are becoming increasingly common in cities (Watson et al., 2020; Marshall et al., 2023), should be promoted. More generally, the importance of urban grasslands in contributing to biodiversity conservation and ecosystem services has been neglected (Klaus, 2013). Given the plant diversity observed in urban grasslands, their restoration or establishment should be promoted as a nature-based solution to provide high-quality habitat for animal species. This is particularly important for pollinating arthropods such as Lepidoptera and Hymenoptera, which are also highly affected by urban environments (Kurylo et al., 2020; Horák et al., 2022). In this context, this study contributes to addressing conservation challenges in the urban context by highlighting the importance of not separating grassland management from urban planning considerations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

CG, AB, SA, VB, and HD conceived and designed the study. CG gathered and analyzed the data with the help of SA, AB, VB, and HD. CG, AB, SA, VB, and HD wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was financed by “l’Institut Agro Rennes Angers” and the “Conseil Régional des Pays de la Loire”.

Acknowledgments

We thank M. Durieu, P. Collignon, and F. Glodt for their help in collecting field data and T. Rodier for spatial data analysis. We also thank “Angers Loire Métropole”, “Rennes Métropole”, “Zone Atelier Armorique”, and all stakeholders involved for their help in sampling the sites.

Conflict of interest

Publisher’s note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2023.1151913/full#supplementary-material

References

Albrecht, H., and Haider, S. (2013). Species diversity and life history traits in calcareous grasslands vary along an urbanization gradient. *Biodivers. Conserv.* 22, 2243–2267. doi: 10.1007/s10531-013-0437-0

Aronson, M. F. J., Lepczyk, C. A., Evans, K. L., Goddard, M. A., Lerman, S. B., MacIvor, J. S., et al. (2017). Biodiversity in the city: key challenges for urban green space management. *Front. Ecol. Environ.* 15, 189–196. doi: 10.1002/fee.1480

Aronson, M. F. J., Nilon, C. H., Lepczyk, C. A., Parker, T. S., Warren, P. S., Cilliers, S. S., et al. (2016). Hierarchical filters determine community assembly of urban species pools. *Ecology* 97, 2952–2963. doi: 10.1002/ecy.1535

Bakker, J. P., Elzinga, J. A., and de Vries, Y. (2002). Effects of long-term cutting in a grassland system: perspectives for restoration of plant communities on nutrient-poor soils. *Appl. Veg. Sci.* 5, 107–120. doi: 10.1111/j.1654-109X.2002.tb00540.x

Barbaro, L., Dutoit, T., Anthelme, F., and Corcket, E. (2004). Respective influence of habitat conditions and management regimes on prealpine calcareous grasslands. *J. Environ. Manage.* 72, 261–275. doi: 10.1016/j.jenvman.2004.05.006

Barton, (2009) *MuMIn : multi-model inference*. Available at: http://r-forge.r-project.org/projects/mumin/https://cir.nii.ac.jp/crid/1572824499154168192 (Accessed November 25, 2022).

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Soft.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bertoncini, A., Machon, N., Pavoine, S., and Muratet, A. (2012). Local gardening practices shape urban lawn floristic communities. *Landsc. Urban Plan.* 105, 53–61. doi: 10.1016/j.landurbplan.2011.11.017

Boussard, H., and Baudry, J. (2017). *Chloe4.0: A software for landscape pattern analysis*. https://bagap.rennes.hub.inrae.fr/productions/logiciels.

Braun-Blanquet, J. (1932). *Plant sociology: the study of plant communities. 1st ed* (New York and London: McGraw-Hill).

Buyantuyev, A., and Wu, J. (2010). Urban heat islands and landscape heterogeneity: linking spatiotemporal variations in surface temperatures to land-cover and socioeconomic patterns. *Landscape Ecol.* 25, 17–33. doi: 10.1007/s10980-009-9402-4

Chaudron, C., Chauvel, B., and Isselin-Nondedeu, F. (2016). Effects of late mowing on plant species richness and seed rain in road verges and adjacent arable fields. *Agric. Ecosyst. Environ.* 232, 218–226. doi: 10.1016/j.agee.2016.03.047

Chocholoušková, Z., and Pyšek, P. (2003). Changes in composition and structure of urban flora over 120 years: a case study of the city of Plzeň. *Flora – Morphol. Distribution Func. Ecol. Plants* 198, 366–376. doi: 10.1078/0367-2530-00109

Chollet, S., Brabant, C., Tessier, S., and Jung, V. (2018). From urban lawns to urban meadows: Reduction of mowing frequency increases plant taxonomic, functional and phylogenetic diversity. *Landsc. Urban Plan.* 180, 121–124. doi: 10.1016/j.landurbplan.2018.08.009

Cochard, A., Pithon, J., Braud, F., Beaujouan, V., Bulot, A., and Daniel, H. (2019). Intraspecific trait variation in grassland plant communities along urban-rural gradients. *Urban Ecosyst.* 22, 583–591. doi: 10.1007/s11252-019-0827-5

Cochard, A., Pithon, J., Jagaille, M., Beaujouan, V., Pain, G., and Daniel, H. (2017). Grassland plant species occurring in extensively managed road verges are filtered by urban environments. *Plant Ecol. Divers.* 10, 217–229. doi: 10.1080/17550874.2017.1350764

English, J., Barry, K. E., Wood, E. M., and Wright, A. J. (2022). The effect of urban environments on the diversity of plants in unmanaged grasslands in Los Angeles, United States. *Front. Ecol. Evol.* 10. doi: 10.3389/fevo.2022.921472

Fahrig, L. (2003). Effects of habitat fragmentation on biodiversity. *Annu. Rev. Ecol. Evol. System.* 34, 487–515. doi: 10.1111/j.1461-0248.2010.01559.x

Fahrig, L., Baudry, J., Brotons, L., Burel, F. G., Crist, T. O., Fuller, R. J., et al. (2011). Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. *Ecol. Lett.* 14, 101–112. doi: 10.1111/j.1461-0248.2010.01559.x

Fischer, L., Lippe, M., and Kowarik, I. (2013). Urban land use types contribute to grassland conservation: The example of Berlin. *Urban For. Urban Green.* 12, 263–272. doi: 10.1016/j.ufug.2013.03.009

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., et al. (2005). Global consequences of land use. *Science* 309, 570–574. doi: 10.1126/science.1111772

Gaujour, E., Amiaud, B., Mignolet, C., and Plantureux, S. (2012). Factors and processes affecting plant biodiversity in permanent grasslands. A review. *Agron. Sustain. Dev.* 32, 133–160. doi: 10.1007/s13593-011-0015-3

Géron, C., Lembrechts, J. J., Borgelt, J., Lenoir, J., Hamdi, R., Mahy, G., et al. (2021). Urban alien plants in temperate oceanic regions of Europe originate from warmer native ranges. *Biol. Invasions* 23, 1765–1779. doi: 10.1007/s10530-021-02469-9

Godefroid, S., and Koedam, N. (2007). Urban plant species patterns are highly driven by density and function of built-up areas. *Landsc. Ecol.* 22, 1227–1239. doi: 10.1007/s10980-007-9102-x

Grime, J. P. (2001) *Plant strategies, vegetation processes, and ecosystem properties.* Available at: https://www.cabdirect.org/cabdirect/abstract/20013069580 (Accessed May 17, 2021).

Grime, J. P., Hodgson, J. G., and Hunt, R. (1988). *Comparative Plant Ecology.* (Dordrecht: Springer). doi: 10.1007/978-94-017-1094-7

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., et al. (2008). Global change and the ecology of cities. *Science* 319, 756–760. doi: 10.1126/science.1150195

Habel, J. C., Dengler, J., Janišová, M., Török, P., Wellstein, C., and Wiezik, M. (2013). European grassland ecosystems: threatened hotspots of biodiversity. *Biodivers. Conserv.* 22, 2131–2138. doi: 10.1007/s10531-013-0537-x

Hahs, A. K., McDonnell, M. J., McCarthy, M. A., Vesk, P. A., Corlett, R. T., Norton, B. A., et al. (2009). A global synthesis of plant extinction rates in urban areas. *Ecol. Lett.* 12, 1165–1173. doi: 10.1111/j.1461-0248.2009.01372.x

Herben, T., Klimešová, J., and Chytrý, M. (2018). Philip Grime's fourth corner: are there plant species adapted to high disturbance and low productivity? *Oikos* 127, 1125–1131. doi: 10.1111/oik.05090

Hill, M. O., Roy, D. B., and Thompson, K. (2002). Hemeroby, urbanity and ruderality: bioindicators of disturbance and human impact. *J. Appl. Ecol.* 39, 708–720. doi: 10.1046/j.1365-2664.2002.00746.x

Hooftman, D., Kimberley, A., Cousins, S. A. O., Escribano-Avila, G., Honnay, O., Krickl, P., et al. (2021). Dispersal limitation, eutrophication and propagule pressure constrain the conservation value of Grassland Green Infrastructure. *Biol. Conserv.* 258, 109152. doi: 10.1016/j.biocon.2021.109152

Horák, J., Šafářová, L., Trombik, J., and Menéndez, R. (2022). Patterns and determinants of plant, butterfly and beetle diversity reveal optimal city grassland management and green urban planning. *Urban For. Urban Green.* 73, 127609. doi: 10.1016/j.ufug.2022.127609

Humbert, J.-Y., Pellet, J., Buri, P., and Arlettaz, R. (2012). Does delaying the first mowing date benefit biodiversity in meadowland? *Environ. Evid.* 1, 9. doi: 10.1186/2047-2382-1-9

Jackson, H. B., and Fahrig, L. (2012). What size is a biologically relevant landscape? *Landsc. Ecol.* 27, 929–941. doi: 10.1007/s10980-012-9757-9

Jackson, H. B., and Fahrig, L. (2015). Are ecologists conducting research at the optimal scale? *Glob. Ecol. Biogeogr.* 24, 52–63. doi: 10.1111/geb.12233

Jantunen, J., Saarinen, K., Valtonen, A., and Saarnio, S. (2007). Flowering and seed production success along roads with different mowing regimes. *Appl. Veg. Sci.* 10, 285–292. doi: 10.1111/j.1654-109X.2007.tb00528.x

Johansen, L. Q., Westin, A., Wehn, S., Iuga, A., Ivascu, C. M., Kallioniemi, E., et al. (2019). Traditional semi-natural grassland management with heterogeneous mowing times enhances flower resources for pollinators in agricultural landscapes. *Glob. Ecol. Conserv.* 18, e00619. doi: 10.1016/j.gecco.2019.e00619

Julve, (2015) *Baseflor. Index botanique, écologique et chorologique de la flore de France, version 2015.* Available at: http://perso.wanadoo.fr/philippe.julve/catminat.htm.

Kahmen, S., Poschlod, P., and Schreiber, K.-F. (2002). Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Bio. Conserv.* 104, 319–328. doi: 10.1016/S0006-3207(01)00197-5

Kimberley, A., Hooftman, D., Bullock, J. M., Honnay, O., Krickl, P., Lindgren, J., et al. (2021). Functional rather than structural connectivity explains grassland plant diversity patterns following landscape scale habitat loss. *Landsc. Ecol.* 36, 265–280. doi: 10.1007/s10980-020-01138-x

Klaus, V. H. (2013). Urban grassland restoration: A neglected opportunity for biodiversity conservation. *Restor. Ecol.* 21, 665–669. doi: 10.1111/rec.12051

Klotz, S., Kühn, I., Durka, W., für Naturschutz, D. B., and Briemle, G. (2002). *BIOLFLOR - eine Datenbank mit biologisch-ökologischen Merkmalen zur Flora von Deutschland.* Münster: BfN-Schriftenvertrieb im Landwirtschaftsverl.

Knapp, S., Kühn, I., Bakker, J. P., Kleyer, M., Klotz, S., Ozinga, W. A., et al. (2009). How species traits and affinity to urban land use control large-scale species frequency. *Divers. Distrib.* 15, 533–546. doi: 10.1111/j.1472-4642.2009.00561.x

Knapp, S., Kühn, I., Stolle, J., and Klotz, S. (2010). Changes in the functional composition of a Central European urban flora over three centuries. *Perspect. Plant Ecol. Evol. Syst.* 12, 235–244. doi: 10.1016/j.ppees.2009.11.001

Kowarik, I. (2008). ““On the role of alien species in urban flora and vegetation,”,” in *Urban Ecology: An International Perspective on the Interaction Between Humans and Nature.* Eds. J. M. Marzluff, E. Shulenberger, W. Endlicher, M. Alberti, G. Bradley, C. Ryan, et al (Boston, MA: Springer US), 321–338. doi: 10.1007/978-0-387-73412-5_20

Kurylo, J. S., Threlfall, C. G., Parris, K. M., Ossola, A., Williams, N. S. G., and Evans, K. L. (2020). Butterfly richness and abundance along a gradient of imperviousness and the importance of matrix quality. *Ecol. Appl.* 30, e02144. doi: 10.1002/eap.2144

Leng, X., Musters, C. J. M., and de Snoo, G. R. (2011). Effects of mowing date on the opportunities of seed dispersal of ditch bank plant species under different management regimes. *J. Nat. Conserv.* 19, 166–174. doi: 10.1016/j.jnc.2010.11.003

Lepš, J., and Šmilauer, P. (2003). *Multivariate Analysis of Ecological Data using CANOCO.* (Cambridge: Cambridge University Press). doi: 10.1017/CBO9780511615146

Lindborg, R., Bengtsson, J., Berg, Å., Cousins, S. A. O., Eriksson, O., Gustafsson, T., et al. (2008). A landscape perspective on conservation of semi-natural grasslands. *Agric. Ecosyst. Env.* 125, 213–222. doi: 10.1016/j.agee.2008.01.006

Loos, J., Krauss, J., Lyons, A., Föst, S., Ohlendorf, C., Racky, S., et al. (2021). Local and landscape responses of biodiversity in calcareous grasslands. *Biodivers. Conserv.* 30, 2415–2432. doi: 10.1007/s10531-021-02201-y

Lososová, Z., Chytrý, M., Kühn, I., Hájek, O., Horáková, V., Pyšek, P., et al. (2006). Patterns of plant traits in annual vegetation of man-made habitats in central Europe. *Perspect. Plant Ecol. Evol. Syst.* 8, 69–81. doi: 10.1016/j.ppees.2006.07.001

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., and Makowski, D. (2021). performance: an R package for assessment, comparison and testing of statistical models. *J. Open Source Software* 6, 3139. doi: 10.21105/joss.03139

Marshall, C. A. M., Wilkinson, M. T., Hadfield, P. M., Rogers, S. M., Shanklin, J. D., Eversham, B. C., et al. (2023). Urban wildflower meadow planting for biodiversity, climate and society : An evaluation at King's College, Cambridge. *Ecol. Solut. Evid.* 4, e12243. doi: 10.1002/2688-8319.12243

McDonnell, M. J., and Pickett, S. T. A. (1990). Ecosystem structure and function along urban-rural gradients: an unexploited opportunity for ecology. *Ecology* 71, 1232–1237. doi: 10.2307/1938259

McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience* 52, 883–890. doi: 10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

McKinney, M. L. (2008). Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosyst.* 11, 161–176. doi: 10.1007/s11252-007-0045-4

Mugnai, M., Ferretti, G., Gesuelli, E., Nuti, L., Di Natale, S., Corti, E., et al. (2023). Site dependence of local variations in taxonomic and functional diversity of plant communities in semi-natural dry grasslands. *Plant Ecol.* 224, 95–111. doi: 10.1007/s11258-022-01282-1

Muratet, A., Machon, N., Jiguet, F., Moret, J., and Porcher, E. (2007). The role of urban structures in the distribution of wasteland flora in the greater Paris area, France. *Ecosystem* 10, 661–671. doi: 10.1007/s10021-007-9047-6

Napoleone, F., Giarrizzo, E., and Burrascano, S. (2021). Habitat conservation state and plant diversity respond to different drivers in semi-natural grasslands. *J. Veg. Sci.* 32, e13055. doi: 10.1111/jvs.13055

Norton, B., Bending, G., Clark, R., Corstanje, R., Dunnett, N., Evans, K., et al. (2019). Urban meadows as an alternative to short mown grassland: effects of composition and height on biodiversity. *Ecol. Appl.* 29, 1095–1115. doi: 10.1002/eap.1946

Olsen, S. L., Evju, M., and Endrestøl, A. (2018). Fragmentation in calcareous grasslands: species specialization matters. *Biodivers. Conserv.* 27, 2329–2361. doi: 10.1007/s10531-018-1540-z

Parr, T. W., and Way, J. M. (1988). Management of roadside vegetation: the long-term effects of cutting. *J. Appl. Ecol.* 25, 1073–1087. doi: 10.2307/2403767

Peng, Y., Mi, K., Wang, H., Liu, Z., Lin, Y., Sang, W., et al. (2019). Most suitable landscape patterns to preserve indigenous plant diversity affected by increasing urbanization: A case study of Shunyi District of Beijing, China. *Urban Forest. Urban Greening* 38, 33–41. doi: 10.1016/j.ufug.2018.11.004

Pithon, J. A., Duflot, R., Beaujouan, V., Jagaille, M., Pain, G., and Daniel, H. (2021). Grasslands provide diverse opportunities for bird species along an urban-rural gradient. *Urban Ecosyst.* 24, 1281–1294. doi: 10.1007/s11252-021-01114-6

Pyšek, P. (1995). "Approaches to studying spontaneous settlement flora and vegetation in central Europe: A review", in *Urban Ecology As the Basis of Urban Planning.* Ed. H. Sukopp, M. Numata and A. Huber (Amsterdam: SPB Academic Publishing), 23–29.

Pyšek, P. (1998). Alien and native species in Central European urban floras: a quantitative comparison. *J. Biogeogr.* 25, 155–163. doi: 10.1046/j.1365-2699.1998.251177.x

Roy, D. B., Hill, M. O., and Rothery, P. (1999). Effects of urban land cover on the local species pool in Britain. *Ecography* 22, 507–517. doi: 10.1111/j.1600-0587.1999.tb01279.x

Rudolph, M., Velbert, F., Schwenzfeier, S., Kleinebecker, T., and Klaus, V. H. (2017). Patterns and potentials of plant species richness in high- and low-maintenance urban grasslands. *Appl. Veg. Sci.* 20, 18–27. doi: 10.1111/avsc.12267

Schmucki, R., Reimark, J., Lindborg, R., and Cousins, S. A. O. (2012). Landscape context and management regime structure plant diversity in grassland communities. *J. Ecol.* 100, 1164–1173. doi: 10.1111/j.1365-2745.2012.01988.x

Sehrt, M., Bossdorf, O., Freitag, M., and Bucharova, A. (2020). Less is more! Rapid increase in plant species richness after reduced mowing in urban grasslands. *Basic Appl. Ecol.* 42, 47–53. doi: 10.1016/j.baae.2019.10.008

Smart, S. M., Bunce, R. G. H., Marrs, R., LeDuc, M., Firbank, L. G., Maskell, L. C., et al. (2005). Large-scale changes in the abundance of common higher plant species across Britain between 1978, 1990 and 1998 as a consequence of human activity: Tests of hypothesised changes in trait representation. *Bio. Conserv.* 124, 355–371. doi: 10.1016/j.biocon.2004.12.013

Smith, L. M., and Haukos, D. A. (2002). Floral diversity in relation to playa wetland area and watershed disturbance. *Conserv. Bio.* 16, 964–974. doi: 10.1046/j.1523-1739.2002.00561.x

Smith, R. S., Shiel, R. S., Millward, D., and Corkhill, P. (2000). The interactive effects of management on the productivity and plant community structure of an upland meadow: an 8-year field trial. *J. Appl. Ecol.* 37, 1029–1043. doi: 10.1046/j.1365-2664.2000.00566.x

Smith, R. S., Shiel, R. S., Millward, D., Corkhill, P., and Sanderson, R. A. (2002). Soil seed banks and the effects of meadow management on vegetation change in a 10-year meadow field trial. *J. Appl. Ecol.* 39, 279–293. doi: 10.1046/j.1365-2664.2002.00715.x

Socher, S. A., Prati, D., Boch, S., Müller, J., Klaus, V. H., Hölzel, N., et al. (2012). Direct and productivity-mediated indirect effects of fertilization, mowing and grazing on grassland species richness. *J. Ecol.* 100, 1391–1399. doi: 10.1111/j.1365-2745.2012.02020.x

Sodhi, N. S., Koh, L. P., Peh, K. S.-H., Tan, H. T. W., Chazdon, R. L., Corlett, R. T., et al. (2008). Correlates of extinction proneness in tropical angiosperms. *Divers. Distrib.* 14, 1–10. doi: 10.1111/j.1472-4642.2007.00398.x

Tautenhahn, S., Heilmeier, H., Götzenberger, L., Klotz, S., Wirth, C., and Kühn, I. (2008). On the biogeography of seed mass in Germany – distribution patterns and environmental correlates. *Ecography* 31, 457–468. doi: 10.1111/j.0906-7590.2008.05439.x

Ter Braak, C. J. F. (1986). Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67, 1167–1179. doi: 10.2307/1938672

Ter Braak, C. J. F., and Smilauer, P. (2012). *CANOCO reference manual and user's guide : Software for ordination (version 5.0)*. Ithaca USA: Microcomputer Power.

Thompson, K., and Jones, A. (1999). Human population density and prediction of local plant extinction in Britain. *Conserv. Biol.* 13, 185–189. doi: 10.1046/j.1523-1739.1999.97353.x

Threlfall, C. G., Ossola, A., Hahs, A. K., Williams, N. S. G., Wilson, L., and Livesley, S. J. (2016). Variation in vegetation structure and composition across urban green space types. *Front. Ecol. Evol.* 4. doi: 10.3389/fevo.2016.00066

Tison, J.-M., and de Foucault, B. (2014). *Flora Gallica: Flore de France* (Mèze: Biotope Editions).

Vallet, J., Daniel, H., Beaujouan, V., and Rozé, F. (2008). Plant species response to urbanization: comparison of isolated woodland patches in two cities of North-Western France. *Landscape Ecol.* 23, 1205–1217. doi: 10.1007/s10980-008-9293-9

Van der Maarel, E. (2005). *"Vegetation ecology - an overview,"*. In Vegetation Ecologie, ed. E. van de Maarel (Oxford: Blackwell Publishing), 1–51.

Vega, K. A., and Küffer, C. (2021). Promoting wildflower biodiversity in dense and green cities: The important role of small vegetation patches. *Urban For. Urban Green.* 62, 127165. doi: 10.1016/j.ufug.2021.127165

Watson, C. J., Carignan-Guillemette, L., Turcotte, C., Maire, V., and Proulx, R. (2020). Ecological and economic benefits of low-intensity urban lawn management. *J. Appl. Ecol.* 57, 436–446. doi: 10.1111/1365-2664.13542

Williams, N. S. G., Hahs, A. K., and Vesk, P. A. (2015). Urbanisation, plant traits and the composition of urban floras. *Perspect. Plant Ecol. Evol. Syst.* 17, 78–86. doi: 10.1016/j.ppees.2014.10.002

Williams, N. S. G., Morgan, J. W., McCarthy, M. A., and McDonnell, M. J. (2006). Local extinction of grassland plants: the landscape matrix is more important than patch attributes. *Ecology* 87, 3000–3006. doi: 10.1890/0012-9658(2006)87[3000:LEOGPT]2.0.CO;2

Williams, N. S. G., Morgan, J. W., Mcdonnell, M. J., and Mccarthy, M. A. (2005). Plant traits and local extinctions in natural grasslands along an urban-rural gradient: *Plant extinctions along an urban-rural gradient*. *J. Ecol.* 93, 1203–1213. doi: 10.1111/j.1365-2745.2005.01039.x

Williams, N. S. G., Schwartz, M. W., Vesk, P. A., McCarthy, M. A., Hahs, A. K., Clemants, S. E., et al. (2009). A conceptual framework for predicting the effects of urban environments on floras. *J. Ecol.* 97, 4–9. doi: 10.1111/j.1365-2745.2008.01460.x

Wu, J. (2014). Urban ecology and sustainability: The state-of-the-science and future directions. *Landscape Urban Plann.* 125, 209–221. doi: 10.1016/j.landurbplan.2014.01.018

Yeh, C.-T., and Huang, S.-L. (2009). Investigating spatiotemporal patterns of landscape diversity in response to urbanization. *Landscape Urban Plann.* 93, 151–162. doi: 10.1016/j.landurbplan.2009.07.002

Zeeman, B. J., McDonnell, M. J., Kendal, D., and Morgan, J. W. (2017). Biotic homogenization in an increasingly urbanized temperate grassland ecosystem. *J. Veg. Sci.* 28, 550–561. doi: 10.1111/jvs.12507

Zhao, Y., Liu, Z., and Wu, J. (2020). Grassland ecosystem services: a systematic review of research advances and future directions. *Landscape Ecol.* 35, 793–814. doi: 10.1007/s10980-020-00980-3

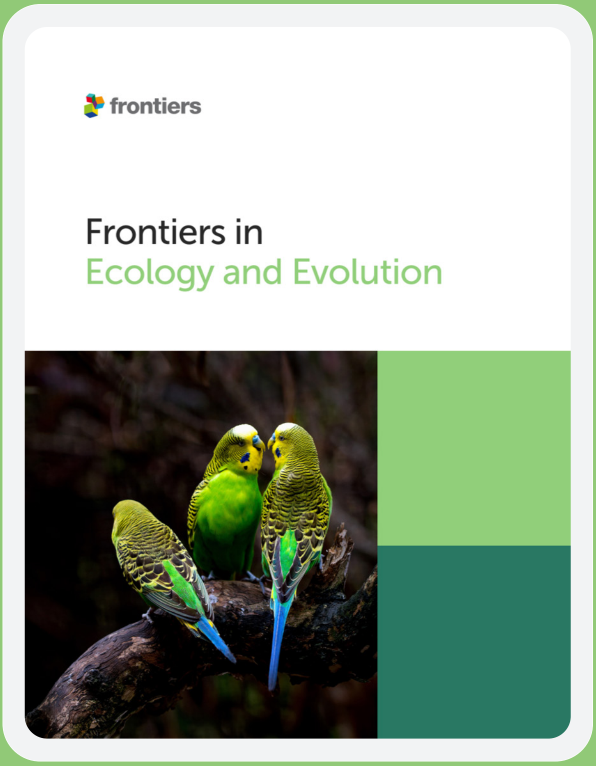
Frontiers in
Ecology and Evolution

Ecological and evolutionary research into our natural and anthropogenic world

This multidisciplinary journal covers the spectrum of ecological and evolutionary inquiry. It provides insights into our natural and anthropogenic world, and how it can best be managed.

Discover the latest Research Topics

See more →



frontiers

Frontiers in
Ecology and Evolution

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact

frontiers | Research Topics