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

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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

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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

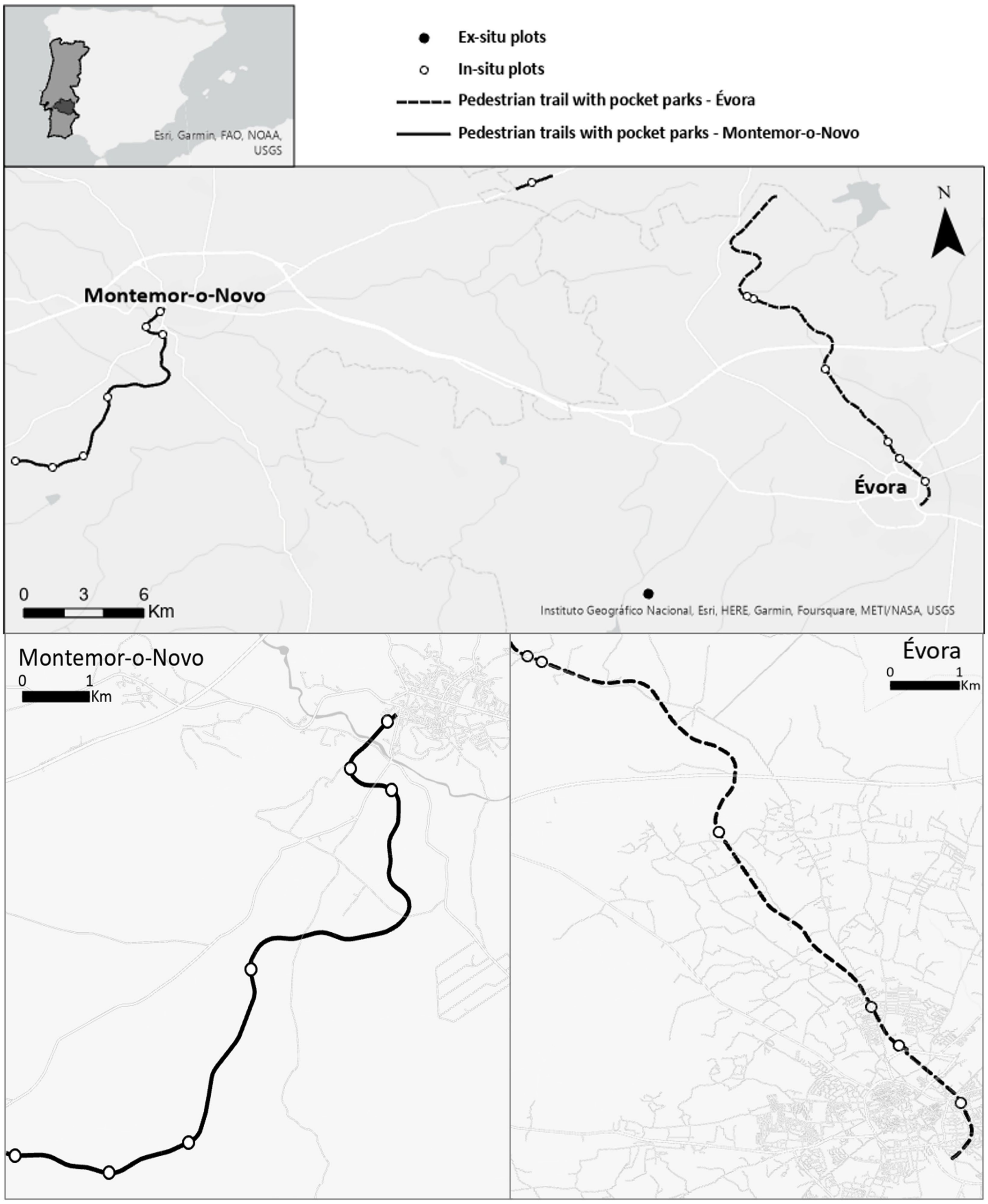

FIGURE 1
Ex-situ and *in-situ* plots installed on the experimental field of the University of Évora and pocket parks distributed throughout pedestrian trails of Évora (six plots) and Montemor-o-Novo (eight plots), respectively.

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 m² 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/m². Seed density followed Hitchmough et al. (2008), according to which sowing with high densities (>1,200 seeds/m²) 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 cm² 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 m²) 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/m² 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 cm² squares (three quadrats/plot evenly distributed throughout the center of the plot). The following

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

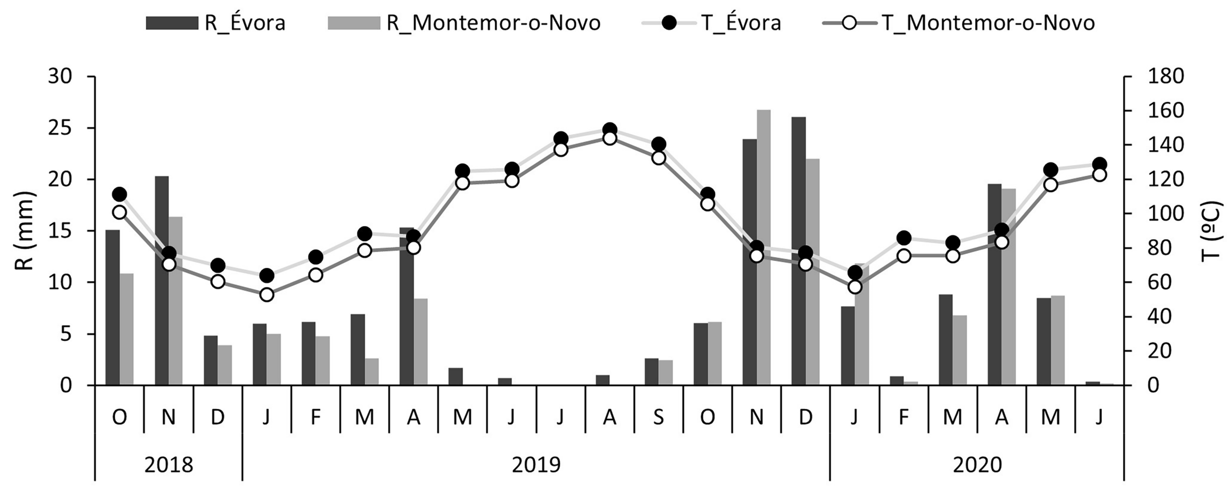

FIGURE 2
Total monthly rainfall (R) and mean monthly temperature (T) recorded accordingly the location of the *in-situ* plots (Évora or Montemor-o-Novo).

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.

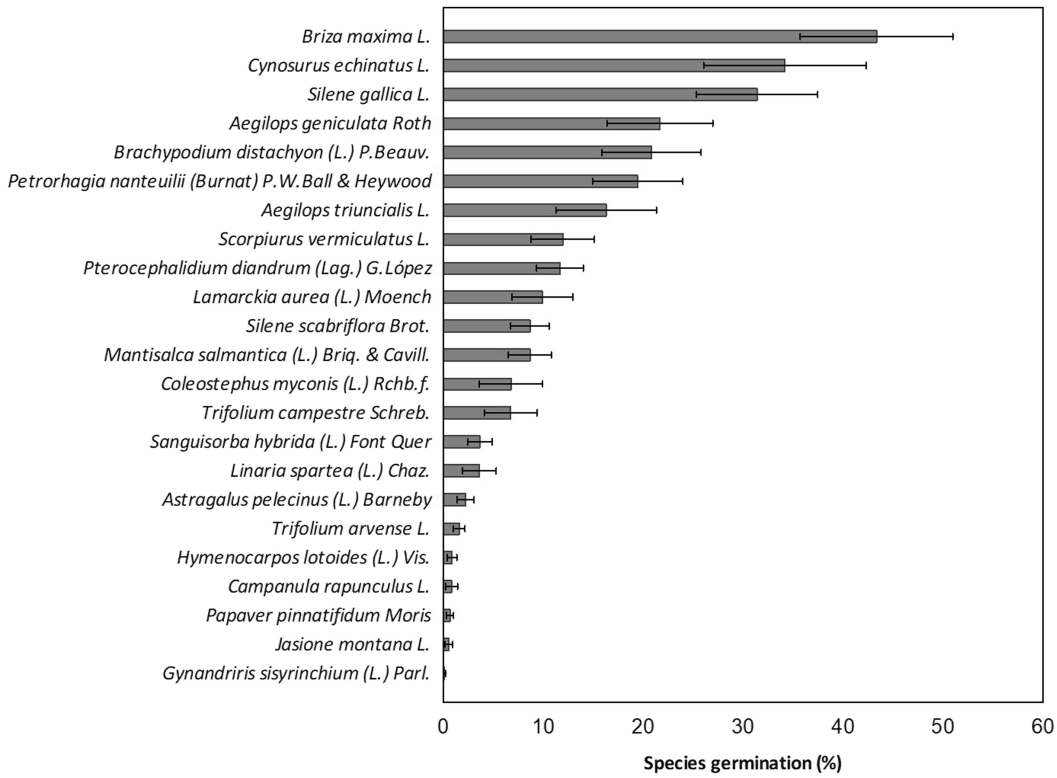

FIGURE 3
Germination percentage (mean±standard error) in 2019 of the seed mix species sown in the *in-situ* plots.

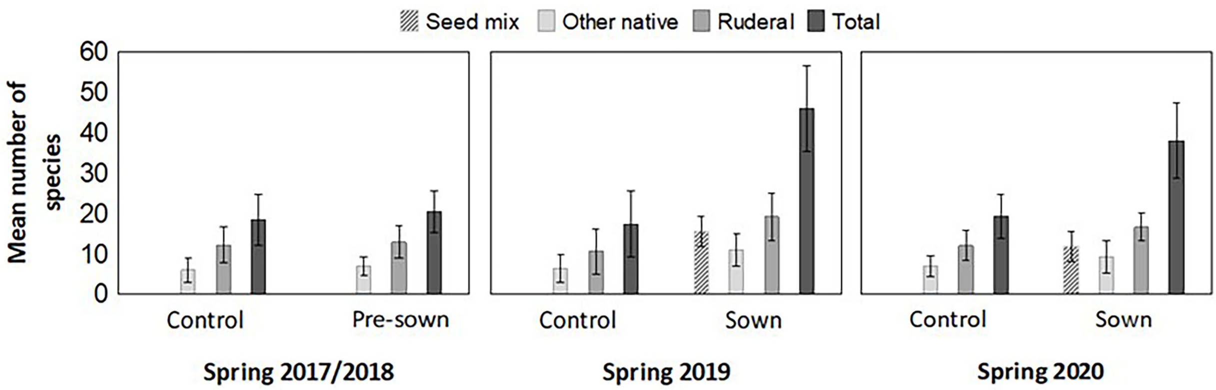

FIGURE 4
Number of species (mean±standard error) in control and *in-situ* sown plots, considering the seed mix species and spontaneous species (other native and ruderal species), and total richness.

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

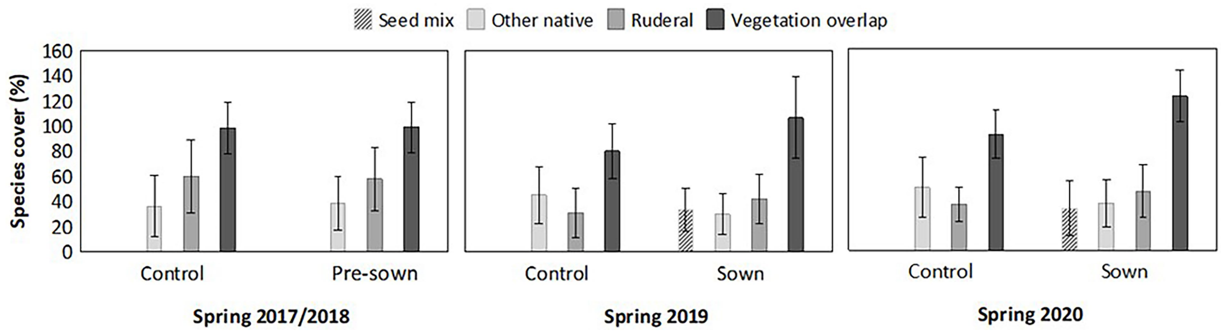

FIGURE 5
Species cover percentage (mean±standard error) in control and *in-situ* sown plots, considering the seed mix species and spontaneous species (other native and ruderal species), and vegetation overlap.

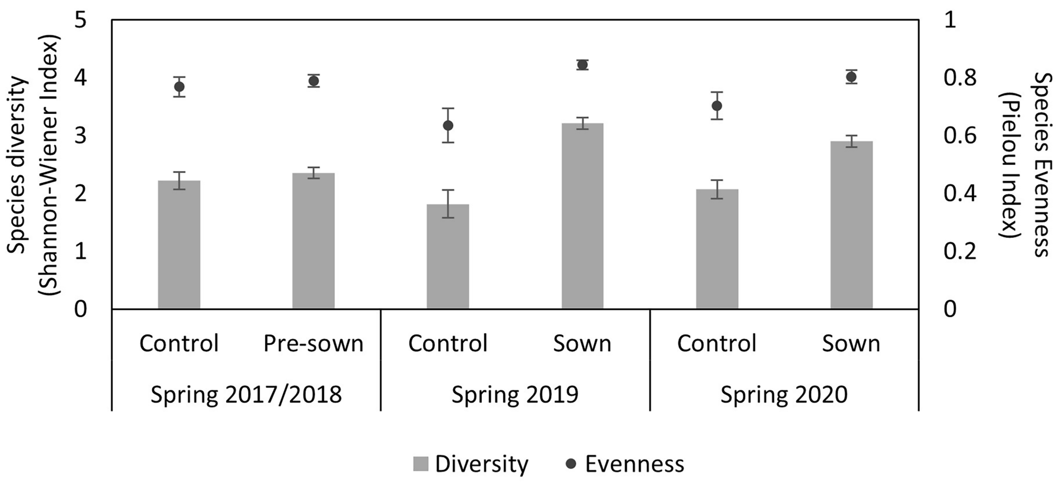

FIGURE 6
Species diversity (Shannon-Wiener index) and Evenness (Pielou Index) of the floristic community (mean±standard error) in control and *in-situ* sown plots.

(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.

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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

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