



OPTIMIZING THE DELIVERY OF MULTIPLE ECOSYSTEM GOODS AND SERVICES IN AGRICULTURAL SYSTEMS

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PUBLISHED IN: Frontiers in Ecology and Evolution and Frontiers in
Environmental Science and Frontiers in Plant Science



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ISSN 1664-8714

ISBN 978-2-88945-296-5

DOI 10.3389/978-2-88945-296-5

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OPTIMIZING THE DELIVERY OF MULTIPLE ECOSYSTEM GOODS AND SERVICES IN AGRICULTURAL SYSTEMS

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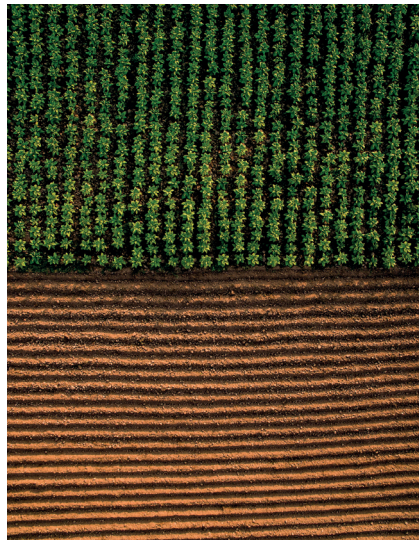
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Agricultural land is subjected to a variety of societal pressures, as demands for food, animal feed, and biomass production increase, with an added requirement to simultaneously maintain natural areas and mitigate climatic and environmental impacts. The biotic elements of agricultural systems interact with the abiotic environment to generate a number of ecosystem functions that offer services benefiting humans across many scales of time and space. The intensification of agriculture generally reduces biodiversity including that within soil, and impacts negatively upon a number of regulating and supporting ecosystem services. There is a global need toward achieving sustainable agricultural systems, as also highlighted in the United Nations Sustainable Development Goals. There is hence a need for management regimes that enhance both agricultural production and the associated provision of multiple ecosystem services.

The articles of this Research Topic enhance our knowledge of how management practices applied to agricultural systems affect the delivery of multiple ecosystem services and how trade-offs between provisioning, regulating, and supporting services can be handled both above- and below-ground. They also show the diversity of topics that need to be considered within the framework of ecosystem services delivered by agricultural systems, from knowledge on basic concepts and newly-proposed frameworks, to a focus on specific ecosystem types such as grasslands and high nature-value farmlands, pollinator habitats, and soil habitats. This diversity of topics indicates the need for broader-scope research, integrated with targeted scientific research to promote sustainable agricultural practices and to ensure food security.

Citation: Tsiafouli, M A., Drakou, E G., Orgiazzi, A., Hedlund, K., Ritz, K., eds. (2017). Optimizing the Delivery of Multiple Ecosystem Goods and Services in Agricultural Systems. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-296-5

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Editorial: Optimizing the Delivery of Multiple Ecosystem Goods and Services in Agricultural Systems

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Keywords: soil, soil biodiversity, pollination, scale, conceptual frameworks, perennial crops, high-value farmlands

Editorial on the Research Topic

Optimizing the Delivery of Multiple Ecosystem Goods and Services in Agricultural Systems

OPEN ACCESS

Edited and reviewed by:

P. K. Ramachandran Nair,
University of Florida, United States

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 14 July 2017

Accepted: 31 July 2017

Published: 15 August 2017

Citation:

Tsiafouli MA, Drakou EG, Orgiazzi A,
Hedlund K and Ritz K (2017) Editorial:
Optimizing the Delivery of Multiple
Ecosystem Goods and Services in
Agricultural Systems.
Front. Ecol. Evol. 5:97.
doi: 10.3389/fevo.2017.00097

Agricultural land is subjected to a variety of societal pressures, as demands for food, animal feed, and biomass production increase, with an added requirement to simultaneously maintain natural areas, and mitigate climatic and environmental impacts globally (Tilman et al., 2002; Pretty, 2008; Wang and Swallow, 2016). The biotic elements of agricultural systems interact with the abiotic environment to generate a number of ecosystem functions that offer services benefiting humans across many scales of time and space (Swinton et al., 2007; Power, 2010). The intensification of agriculture, particularly of that founded on fossil-fuel derived inputs, generally reduces biodiversity, including soil biodiversity (Tsiafouli et al., 2015) and impacts negatively upon a number of regulating and supporting ecosystem services (Zhang et al., 2007). There is a global need toward achieving sustainable agricultural systems, highlighted also in the UNs' Sustainable Development Goals, where among their targets they state that by 2030 we should globally "ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality" (UN-DESA/DSD, 2014).

There is hence an evident need for management regimes that enhance both agricultural production and the provision of multiple ecosystem services. The articles of this Research Topic enhance our knowledge of how management practices applied to agricultural systems affect the delivery of multiple ecosystem services and how trade-offs between provisioning, regulating, and supporting ecosystem services can be handled both above- and below-ground, and across multiple scales of space and time. They also show the diversity of topics that need to be considered within the framework of ecosystem services delivered by agricultural systems, from knowledge on basic concepts and newly-proposed frameworks (§1), to a focus on specific ecosystem types such as grasslands and high nature-value farmlands (§2), pollinator habitats (§3), and soil habitats (§4).

CONCEPTUAL AND METHODOLOGICAL FRAMEWORKS—INTEGRATING HUMAN-INDUCED, BIOTIC, AND ABIOTIC PROCESSES ACROSS SCALES

Although the knowledge on management practices and their impacts on the biotic and abiotic components of agricultural landscapes are widely studied, application-oriented and targeted theoretical and methodological frameworks, keep emerging (e.g., Therond et al., 2017). Such frameworks are developed and assessed across a range of spatio-temporal scales to ensure their validity. For instance, Tscharrntke et al. (2005) have highlighted the importance of the landscape-scale approach to investigating effects of agricultural management practices. The series of conceptual and application-oriented articles presented in this Research Topic show how configuration of agricultural land at landscape spatial scales is linked to the optimization of ecosystem service delivery. Starting with a conceptual discussion paper, Ekroos et al. redirect the debate on what is the best practice between “land sparing” and “land sharing,” to a new cross-scale assessment to improve the management of transformed landscapes. They argue that in order to ensure that agricultural systems are able to maximize yields while maintaining a series of ecosystem benefits, a multiple-scale land-sparing practice is required. They propose to apply this larger scale approach either within groups of collaborating farms or at a regional level, while taking into account the trade-offs among scales.

Williams et al. in their opinion paper give an overview of the background knowledge required to apply and use soil functional zone management (SFZM) methods for more sustainable systems of agricultural production. They also present a conceptual framework that can be applied to account for the feedback loops generated in agricultural production. They claim that the SFZM method allows the sustainable management of the soil, in order to provide multiple ecosystem services directly linked to agricultural production, or indirectly supporting it. It also allows to minimize trade-offs among ecosystem services generated by soils and agricultural production. Furthermore, SFZM can lead to productivity optimization. Vinatier et al. in their perspective article propose a spatially-explicit unified conceptual framework that integrates biotic and abiotic processes and human activities. In particular, they propose a shared representation of distinct immobile and mobile (both biotic and abiotic) landscape elements, which allow understanding of complex landscape functioning by different disciplines and the setting of common objects and spatio-temporal process boundaries. Their framework develops a common view of agricultural landscapes, simplifies the representation of the complex system, but leaves the possibility to include current modeling strategies specific to biotic or abiotic disciplines.

Schulte et al. in their hypothesis and theoretical article set a Functional Land Management framework for policy-making. Within this framework demands of food security and environmental sustainability are met by incentivizing land use and soil management practices that selectively augment specific soil functions, such as provision of food, fiber and fuel, water

purification and carbon sequestration, and biodiversity support, where required. Using Ireland as a case study they show that demands for contrasting soil functions may apply to very different spatial scales, and require local or regional/national scale management. For optimization they refer to 14 policy and market instruments that are available in the European Union and outline the merit in adapting existing governance instruments by facilitating differentiation between soils and landscapes.

In a concept-and-application paper, Marton et al. explain how a “contract rearing system” could be adopted for sharing labor between the labor-intensive dairy production system in the lowlands and the less-labor-intensive farming system in the mountains. This sharing of intensity helps maintain attractive landscapes (i.e., a cognitive function) and fosters their high nature value, while reducing environmental impacts of agricultural production.

GRASSLANDS, PERENNIAL, AND HIGH NATURE-VALUE FARMLANDS

The advantages of farmlands involving perennial crops and grasslands vs. annual crops is a topic of broad discussion and research over the past decade (e.g., Ferchaud et al., 2016; Miller et al., 2016; Vico et al., 2016). Several articles in this Research Topic focus on how different types of farmlands and other land uses are providing multiple long-term ecosystem services. In search of grassland vegetation restoration for preventing soil erosion and desertification, Zhang et al. investigate the results of land enclosure in the Loess Plateau of northwestern China. They demonstrate that a moderate amount of litter favors seedling emergence, whereas continually increasing litter diminishes the phenomenon. They suggest that a moderate utilization of litter can lead to moderate disturbance, which favors downward movement of seeds and accelerates vegetation restoration. They conclude that litter amount can serve as a guide for monitoring and managing grassland ecosystems, as it is an indicator of ecosystem processes that are essential for biodiversity conservation and restoration.

On perennial systems, Winkler et al. explore the multifunctional role of vineyard ecosystems as agricultural landscapes. Through a literature review, they identify that the multi-functionality of vineyards has rarely been explored or taken into account into management. They provide a detailed analysis/description of the different ecosystem services provided by vineyards and conclude that there are very few cases in which such services are considered in the management of vineyards, mostly linked to cultivated crops and sequestration. Finally, authors call for more research and practice that applies the ecosystem service approach to vineyard systems. Gardi et al. investigate soil carbon (C) storage in high nature-value farmlands of Europe. By considering soil carbon content as a proxy for storage they show that the C content is higher in high nature-value farmlands than in conventional ones, with the amount of C strongly affected by the type of land use and the geographic region. They also discuss limitations

in the classification and mapping of agricultural systems at continental scale. In order to mitigate these limitations, they suggest first to evaluate soil C content at a more detailed scale as a function of different types of land/agricultural management under different environmental conditions, and then upscale the information.

POLLINATOR HABITATS

Pollination is a key ecosystem function of insects targeting pollinator-dependent plants. The pollinator–plant interaction can increase the productivity of many agricultural systems thereby contributing to societal well-being (Klein et al., 2007; Lautenbach et al., 2012; Garibaldi et al., 2014; IPBES, 2016). Four articles covering this Research Topic highlight the significance of pollination and outline ways to preserve and enhance pollinators and their habitats in different spatial scales.

Sidhu and Joshi in their opinion article suggest methods to optimize wildflower pollinator habitats in agricultural farmland to benefit from multiple ecosystem services. The authors consider factors such as complementary composition of wildflower availability and crop bloom period pollination requirements and highlight the importance of pollinator habitats establishment in simple landscapes with fragmented resources. The factors considered might hinder farmers to establish pollinator habitats. In order to increase their acceptance and use, they recommend focusing more on the role of pollinator habitats in supporting on-farm pest control, than biodiversity conservation in general. Klatt et al. in their opinion article argue how restriction of neonicotinoid insecticides in the European Union might provide benefits but also risks to bees and pollination services. As the use of pesticides is an integral part of contemporary agriculture, the authors claim that neonicotinoid restrictions will lead farmers to find suitable alternatives. Knowledge about the general impact of insecticides on pollinators is still limited, especially considering long-term effects and pollinator species others than bees. They conclude that an expanded scientific evidence base is needed to assess the risks and benefits of alternative pest management strategies.

Joshi et al. specifically explore the factors that drive pollinator visitation in apple orchards and associate it with landscape patterns in order to identify the best landscape configuration to ensure high pollination rates for apple trees. They focus on heterogeneous landscapes surrounding apple orchards, and the impact they have on the frequency of several pollinator species. From their field measurements (Pennsylvania, USA) it appears that proximity to unmanaged habitat and low degree of heterogeneity were the principal prevailing factors affecting the pollinator-visitation frequency. Finally, Parejo et al. present a genomic approach, based on molecular markers, for monitoring European honey bee subspecies and, thus, ensuring the delivery of pollination services. Their analysis prescribes a limited panel of single nucleotide polymorphisms (SNPs) which could be used for distinguishing native from non-native honey bee populations and, consequently, establishing preservation actions.

SOIL AND SOIL BIODIVERSITY

Soil ecosystem services generated from soil food webs play a key role in sustainable agricultural systems (Shennan, 2008; De Vries et al., 2013; Ferris and Tuomisto, 2015). Four articles on this Research Topic focus on agricultural practices applied to soil and soil biodiversity toward increasing multi-functionality in agro-ecosystems. Zhang et al. explore the application of a common agricultural practice in China, that of film mulching, as a way to increase water availability for crops and thus to improve production of maize. They applied five different types of mulching and estimated how each affected topsoil temperature, soil water storage and maize yield and also the corresponding economic revenues. The consecutive film-mulched ridge method proved to be the one producing the highest yield for maize fields in such semi-arid regions.

Koskey et al. examined the effectiveness of native rhizobia isolates in enhancing nitrogen fixation and yields of climbing beans in greenhouse and field experiments in Kenya. They found four native rhizobia isolates to show higher symbiotic efficiency compared to commercial inoculants in both the considered bean varieties. One of the inoculants led to up to 90% highest seed yield compared to non-inoculated control and up to 30% increase over commercial inoculants. They conclude that within the framework of enhancing delivery of agroecosystem services, such as nitrogen fixation and bean production, characterization and mapping of native isolates is imperative to develop effective and affordable commercial inoculants. This demonstrates that some strategies based upon local circumstances are likely to be more effective than those that attempt large-scale unified solutions that may require other interventions to compensate for the inherent diversity in systems at such local levels. The scale of such “locality” may also vary according to circumstances.

The need for conservation actions to support the ecosystem services provided by different arthropods is the common denominator of the manuscripts by Birkhofer et al. and de Groot et al. The former article investigates the effects of different land-use intensities on soil mites, collembolans, myriapods and the functions that they sustain in grasslands, from decomposition to control of belowground pests. The study confirms the potential of both biodiversity and food web analyses to assess the impact of human interventions on ecosystem services delivered by soil fauna. De Groot et al. examined the results of land management extensification. They specifically studied the succession of mite communities following conversion of arable land to grassland in a chronosequence representing a 29-year long period. They found that diversity and biomass of mites steadily rose over time since the conversion. Consequently, nutrient cycling increased and the suppression of potential pests improved. They discuss the relevance of these ecosystem services in extensively managed grasslands and suggest including grasslands for one or more years in agricultural rotational schemes to ensure a better provision of the considered ecosystem services. Finally, the meta-analysis of Trivedi et al. provide evidence that conversion from natural grasslands to agriculture cause community scale trends

of microbial biodiversity across global biomes. They propose that microbial abundance may serve as an indicator for changing soil health as it is more responsive to land use change than other physical and chemical soil factors. This knowledge can be used to facilitate decision making for soil health and soil biodiversity.

CONCLUSIONS

Collectively, the papers in this Research Topic consider very different concepts of what it takes to optimize the delivery of multiple ecosystem services in agricultural systems: they address basic concepts, encompass small to large scales, changes in time and space, soil management, and soil biodiversity to pollination. They essentially form a scientific ensemble that helps evaluate the impact of human pressures on the provision of ecosystem services and nurture the development of measures for a more sustainable management of agricultural areas. The publication of this volume comes at a key moment in which the delivery of

ecosystem services by agricultural systems is of high importance (IPBES, 2016) and the need to achieve the UN Sustainable Development Goals for 2030 (UN-DESA/DSD, 2014) becomes an urgent issue to be addressed. We aspire that this collective work will inform and stimulate more studies on this Research Topic the coming years. Whilst it is sometimes a cliché to state that “further research is needed....,” this is certainly the case here, fact also highly supported by the great amount of conceptual papers (§1). The ensemble of papers shows that whilst generalized theories can be established, there is also a high degree of context-specificity in optimal solutions in different circumstances. We argue that broader-scope research (including development of new concepts and frameworks) needs to be integrated with targeted scientific research to promote sustainable agricultural practices, ensure food security and decrease hunger and poverty.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest Statement: 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.

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Sparing Land for Biodiversity at Multiple Spatial Scales

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OPEN ACCESS

Edited by:

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 28 August 2015

Accepted: 08 December 2015

Published: 05 January 2016

Citation:

Ekroos J, Ödman AM,
Andersson GKS, Birkhofer K,
Herbertsson L, Klatt BK, Olsson O,
Olsson PA, Persson AS, Prentice HC,
Rundlöf M and Smith HG (2016)
Sparing Land for Biodiversity at
Multiple Spatial Scales.
Front. Ecol. Evol. 3:145.
doi: 10.3389/fevo.2015.00145

A common approach to the conservation of farmland biodiversity and the promotion of multifunctional landscapes, particularly in landscapes containing only small remnants of non-crop habitats, has been to maintain landscape heterogeneity and reduce land-use intensity. In contrast, it has recently been shown that devoting specific areas of non-crop habitats to conservation, segregated from high-yielding farmland (“land sparing”), can more effectively conserve biodiversity than promoting low-yielding, less intensively managed farmland occupying larger areas (“land sharing”). In the present paper we suggest that the debate over the relative merits of land sparing or land sharing is partly blurred by the differing spatial scales at which it is suggested that land sparing should be applied. We argue that there is no single correct spatial scale for segregating biodiversity protection and commodity production in multifunctional landscapes. Instead we propose an alternative conceptual construct, which we call “multiple-scale land sparing,” targeting biodiversity and ecosystem services in transformed landscapes. We discuss how multiple-scale land sparing may overcome the apparent dichotomy between land sharing and land sparing and help to find acceptable compromises that conserve biodiversity and landscape multifunctionality.

Keywords: central-place foraging, metapopulation, protected area, habitat patch network, landscape complementation, landscape mosaic

INTRODUCTION

Agricultural intensification and expansion have caused major losses of global biodiversity leading to fundamental trade-offs between food production and species conservation (Mattison and Norris, 2005). Strategies that preserve landscape heterogeneity and reduce agricultural land-use intensity are commonly used to conserve farmland biodiversity (Benton et al., 2003) and promote multifunctional landscapes (Foley et al., 2005). However, less intensive agricultural practices, such as organic farming, are often associated with reduced yields (Seufert et al., 2012; but see Ponisio et al., 2015), and in addition, it is not clear if such schemes generate aggregated biodiversity benefits at larger spatial scales (Kleijn et al., 2011; Birkhofer et al., 2014; Schneider et al., 2014). Recent research suggests that agricultural production needs to increase dramatically to feed globally increasing human populations, coupled with expected dietary shifts (Valin et al., 2014). It has been suggested that increased production could be achieved by closing yield gaps by, for example, increasing yields on existing farmland through optimized inputs of mineral nutrients (Foley et al., 2011; Mueller et al., 2012). However, other studies emphasize that, rather than focussing

solely on increased production, there is a need to consider alternative ways of improving food security: reduction of food waste, reduction of the demand for livestock feed, and achieving an equitable distribution of the food that is currently produced (Fischer et al., 2014; Loos et al., 2014). Nevertheless, the conflicts between biodiversity protection and food production appear to be stronger than ever before.

By conceptualizing two contrasting land-use scenarios; *land sparing* and *land sharing*, it has been suggested that trade-offs between biodiversity and food production are more likely to be alleviated by the spatial segregation of food production and conservation, which would allow for higher yields within smaller shares of lands (Green et al., 2005; Phalan et al., 2011a,b; Balmford et al., 2012). A land-sparing approach has been suggested to be particularly suitable in the tropics, where forests with high biodiversity values are threatened by expanding agriculture (Ramankutty and Rhemtulla, 2012). A complete segregation of land areas devoted to commodity production and biodiversity protection has nevertheless been criticized for two ecological reasons. First, the fact that a large proportion of species of conservation concern occur outside protected areas, also necessitates conservation strategies within production land (including both farmland and managed forests; Rodrigues et al., 2004; Palomo et al., 2014; Troupin and Carmel, 2014). Second, segregation strategies may increase negative off-site effects from intensive agriculture (Foley et al., 2005) and erode ecosystem services within agricultural systems—ultimately affecting yields and hindering the development of ecologically sustainable, high-yielding farming systems (Bommarco et al., 2013).

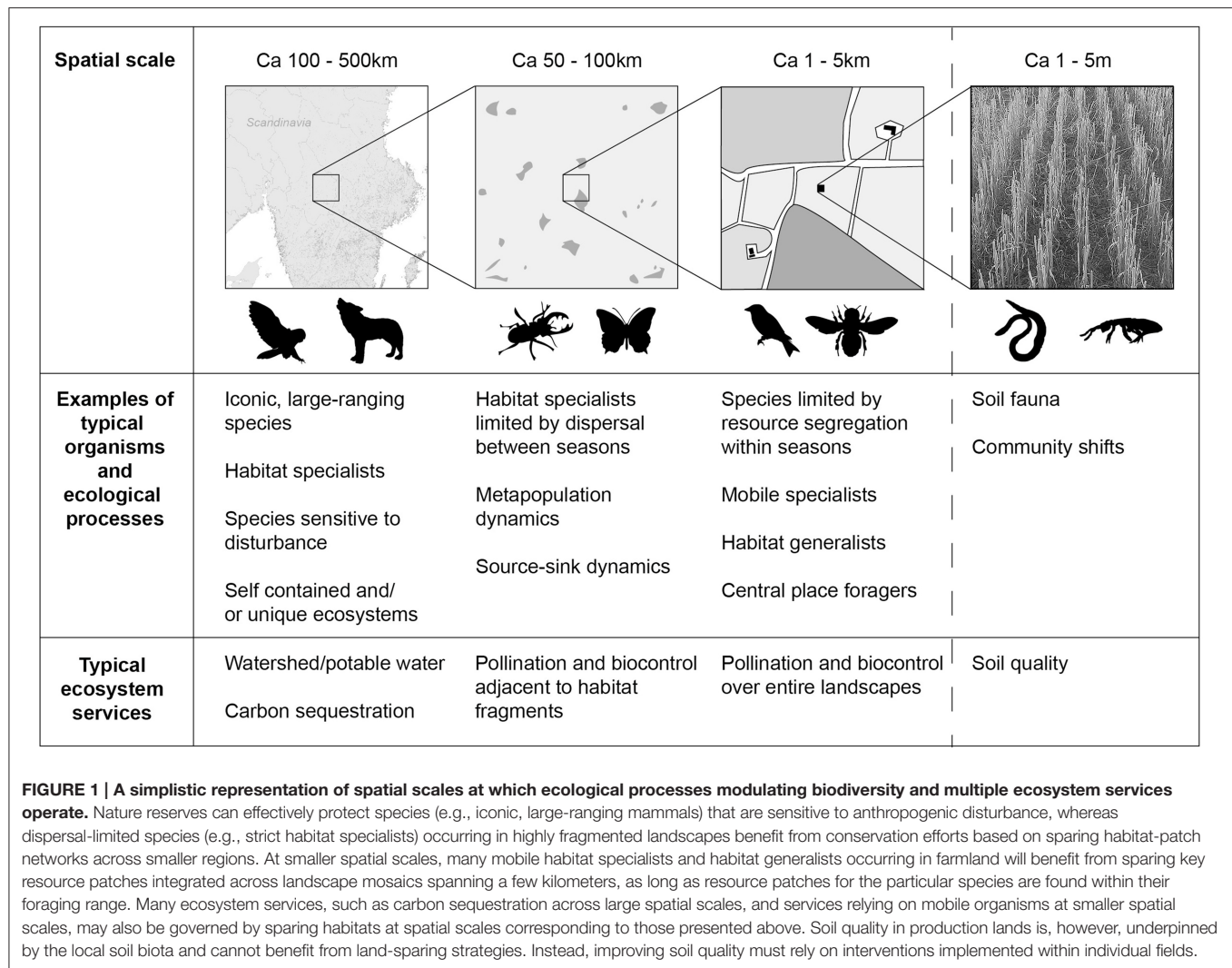
Land sparing and land sharing are broadly defined as, respectively, segregating or integrating commodity production (e.g., agricultural production) and biodiversity protection. The production side in this relationship has been conceptualized by comparing landscapes along landscape-wide gradients of agricultural yields and baseline habitats with no yields (Phalan et al., 2011a,b), by exemplifying “land sparing” and “land sharing” with particular habitat types along land-use intensity gradients (e.g., Egan and Mortensen, 2012; Quinn et al., 2012), or by combinations of these approaches (Gabriel et al., 2013; Gilroy et al., 2014). However, particularly in relation to the latter two approaches, there is a wide variation in definitions of what constitutes spared land. While some researchers argue that only natural or near-natural habitats should be used to represent land-sparing strategies (Phalan et al., 2011a), others have used grazed grasslands (Dotta et al., 2015; Kamp et al., 2015), or managed grasslands, set-asides, and field boundaries (Quinn et al., 2012; Gabriel et al., 2013), to represent land sparing. A similar variability in definitions also complicates the interpretation of what could constitute land sharing, which has been exemplified by organic farming (Hodgson et al., 2010; Gabriel et al., 2013), shade coffee (Chandler et al., 2013), silvopastures (Macchi et al., 2013), or landscapes with low human population densities (Chapron et al., 2014). In addition, while some researchers argue that land sparing must be carried out across large areas if it is to benefit biodiversity conservation (Phalan et al., 2011a), others have advocated that land sparing can be applied successfully at relatively small spatial scales (Gabriel et al., 2013).

In the present paper we suggest that the debate over the relative merits of land sparing or land sharing (Phalan et al., 2011a; Tscharntke et al., 2012a; Fischer et al., 2014) is partly blurred by the differing spatial scales at which it is suggested land sparing should be applied. Neither sparing nor sharing is conceptually tied to a particular scale, creating a definitional gray zone (Fischer et al., 2014) and making it challenging to generalize outcomes between land sparing and land sharing across different contexts. Despite this, it is clear that conservation efforts are needed at multiple spatial scales both in general (Lindenmayer et al., 2006) and, more specifically in agricultural landscapes (Benton et al., 2003; Fischer et al., 2008).

To allow a discussion of multiple scale sparing strategies, we explicitly define land sparing as delimiting habitats known or supposed to be important for biodiversity and land primarily devoted to commodity production, irrespective of the spatial scale at which this is carried out. The spared land may thus constitute larger reserves (Phalan et al., 2011b), more fine-grained habitat fragments (Hodgson et al., 2010), or at even smaller spatial scales, field boundaries (Gabriel et al., 2013). The framework of land sparing vs. land sharing can be applied for any production system (Edwards et al., 2014; Paul and Knoke, 2015; Stott et al., 2015), but here we focus on trade-offs between agricultural production and biodiversity conservation. We define land sharing as any intervention intended to benefit biodiversity by reducing in-field agricultural intensity, for example by reducing stocking rates in pastures and the use of agro-chemicals in arable fields, which therefore reduces yields compared to a baseline of intensive agricultural production. However, we are fully aware that in practice there may be cases where it is debatable whether a specific measure constitutes sharing or sparing (e.g., integrated pest management, when long-term set-asides are established on arable land, or when spared land needs low-intensive agricultural management to maintain biodiversity). We highlight the need for maintaining biodiversity-friendly habitats over a hierarchy of spatial scales (**Figure 1**) in order to conserve biodiversity and to benefit ecosystem services relevant for agricultural production (Bommarco et al., 2013; Ekroos et al., 2014). Finally, we discuss whether multiple-scale land sparing is sufficient to reach these goals, or whether land sharing may additionally be needed in some circumstances.

BIODIVERSITY CONSERVATION ACROSS MULTIPLE SPATIAL SCALES

Conservation at different spatial scales is necessary because conservation has multiple objectives, which range from the conservation of wide-ranging iconic species to the integration of functional biodiversity within production landscapes (Mace et al., 2012). Traditional conservation has a focus on sparing specific areas that are known to be important for species in need of conservation efforts, or on distinct habitat types that form the basis for current biodiversity protection schemes (Secretariat of the Convention of Biological Diversity, 2008).



However, conservation efforts at large spatial scales need to be complemented by conservation efforts at smaller, more fine-grained, spatial scales. In human-modified landscapes the opportunities for conservation are set by historical land-use, and opportunities for sparing large areas of land may not be a practical alternative. While conservation at large spatial scales creates areas with a high degree of ecological integrity, conservation at smaller spatial scales may facilitate the dispersal of organisms between fragmented habitats (Honnay et al., 2002), or provide complementary resources for mobile species (Smith et al., 2014). Below we therefore also illustrate how sparing at two smaller spatial scales affect two distinct ecological processes of importance for biodiversity conservation. At regional scales, metapopulation dynamics govern spatially structured populations, because local populations face significant risk of extinction, whereas at smaller spatial scales, processes affecting regular foraging movements of animals in landscape mosaics, determine population persistence (see Figure 1).

SPARING HABITAT AT LARGE SPATIAL SCALES: CONTIGUOUS PROTECTED AREAS

Protected areas constitute the backbone of traditional nature conservation efforts (Baudron and Giller, 2014), with over 12% of the world's total land area currently under some form of formal protection (Joppa and Pfaff, 2011). The argument for focussing conservation efforts on large, contiguous nature reserves is supported by ecological theory: the concept of minimum viable population size suggests that the success of an effort to conserve a threatened species is likely to increase with increasing population size (Frankham et al., 2014), while the species-area relationship predicts that species richness will increase with increasing area and increasing habitat diversity (Rosenzweig, 1995). In addition, the ecological integrity of a reserve is more likely to remain intact with increasing reserve area and habitat diversity (Schwartz, 1999). However, as further discussed below, a network of small reserves with a small total area may also capture a high overall

level of diversity in some cases (Tscharntke et al., 2012b). Such observations have led some researchers to question the anticipated strong negative effects of habitat fragmentation on biodiversity (Fahrig, 2013), whereas others point out that simple estimates of species richness provide a poor basis for effective biodiversity conservation (Phalan et al., 2011a; Hanski, 2015).

To efficiently protect species within protected areas, these should be large enough to maintain ecological integrity and cover representative habitat types over large geographic areas. Protected areas have historically not been designated on the basis of ecological integrity, but instead on factors such as scenic, recreational or hunting value, or the protection of large-ranging iconic species (Pressey, 1994; Scott et al., 2001). Furthermore, because of the high opportunity costs of conservation in production landscapes, most protected areas lie in regions with low productivity and, as a consequence, these protected areas are likely to target only a subset of the overall regional diversity (Joppa and Pfaff, 2011). In Europe the Habitats Directive (European Commission, 1992) and the Birds Directive (European Commission, 2009) forms a binding legislative framework for creating a network of protected areas identified across large geographic scales (the Natura 2000 network). The Natura 2000 network has been found to perform better compared to nationally designated protected areas (Sánchez-Fernández et al., 2013), but substantial gaps have also been identified (D'Amen et al., 2013; Maiorano et al., 2015; Sánchez-Fernández and Abellán, 2015). In addition, although protected areas may be large enough to contain multiple habitats and ecosystems, they often do not include all necessary habitat elements required to maintain natural disturbance regimes, nutrient flows, organism movements, and population processes within them (Hansen and DeFries, 2007; Wiens, 2009).

SPARING HABITAT AT REGIONAL SCALES: HABITAT PATCH NETWORKS

Strict habitat specialists are dependent on a particular type of habitat and, because of land-use change, many such habitats are currently highly fragmented (Hanski, 2005; Tscharntke et al., 2012b). Individual habitat fragments are often not large enough to sustain viable populations, particularly in the absence of immigration. In such situations, protecting networks of remaining habitat fragments across entire regions becomes essential for the maintenance of long-term population viability. Rare but recurrent dispersal events between habitat fragments are a typical feature of metapopulation dynamics (Fronhofer et al., 2012) and may play a particularly important role for habitat specialists that occur in highly fragmented landscapes, such as semi-natural grassland in Central Europe (Tscharntke et al., 2012b) or old-growth forests (Hanski, 2005). A metapopulation can form when the environment is structurally highly variable, and when between-patch movement is possible, yet much reduced by dispersal barriers (Fronhofer et al., 2012). The best-known empirical example of a metapopulation is provided by the Glanville Fritillary (*Melitaea cinxia*) on the Åland Islands, where the butterfly occurs in highly fragmented dry grasslands.

The ~1600 grassland fragments are located within an area of roughly $75 \times 50 \text{ km}^2$, covering only around 2 km^2 altogether (Ojanen et al., 2013).

Poor dispersal, for example limited seed dispersal in plants, may reduce the ability of species to colonize available patches of suitable habitat (Riibak et al., 2015). For example, plant population sizes and levels of genetic diversity are often positively related to historically less fragmented landscapes. Grassland plants are therefore often characterized by an extinction debt leading to further losses of species, even though there is no further habitat fragmentation (Kuussaari et al., 2009). Sparing habitat fragments across regions in a way that takes into account the historical distribution of habitat fragments as well as current dispersal probabilities between local populations may therefore be critically important to maximize and maintain overall diversity. In Europe, grassland habitat specialists are often restricted to sites that have had a prolonged history of grazing and nutrient-removal (Purschke et al., 2013). Such sites, which have had hundreds of years of grazing continuity, can therefore not be substituted by young patches of nutrient-rich grassland habitat.

SPARING HABITATS IN LANDSCAPE MOSAICS

Many animals, including species that are relatively specialized in their habitat and resource use, are mobile enough to persist within mosaic landscapes that consist of multiple distinct habitat patches, separated by distances of tens to thousands of meters. In some cases organisms have to use resources in multiple patches for populations to be viable (landscape complementation and supplementation *sensu* Dunning et al., 1992). In contrast to habitat patch networks, where migration events between patches are rare because of dispersal limitation, habitat mosaics are characterized by regular movements between habitat patches, either within seasons (e.g., spill-over processes) or within days (e.g., multipatch foraging; Tscharntke et al., 2005; Fahrig et al., 2011; Blitzer et al., 2012; Smith et al., 2014). Many relatively common organisms use multiple habitats that offer complementary or supplementary resources, and therefore perceive landscapes as mosaics consisting of habitat patches varying in quality over space and time (Fahrig et al., 2011; Tscharntke et al., 2012b). The habitat patches of the mosaic landscape are used by animals for separate needs, such as food, shelter, nesting, and/or hibernation (Fahrig et al., 2011). Although the need for between-patch movement may vary over the day or over the season, unconstrained and regular movement is fundamentally important for species to be able to persist in mosaic landscapes.

Daily movements are particularly important for the short-term persistence of central-place foragers, such as birds and nest-building insects, which often feed in multiple habitat patches that together sustain populations through landscape complementation and/or landscape supplementation processes (Olsson and Bolin, 2014; Smith et al., 2014). Bumble bees nesting in semi-natural habitats regularly fly out into the surrounding agricultural landscape to forage in other, more flower rich

habitats (Öckinger and Smith, 2007; Garibaldi et al., 2011; Ekroos et al., 2013). Some species forage over large areas and are thus able to utilize spatially scattered resources, including highly rewarding, but seasonally restricted resources such as mass-flowering crops (Garibaldi et al., 2011; Rundlöf et al., 2014). Therefore, preserving key habitat features offering nesting and feeding sites at a scale within daily or seasonal movement ranges will be important to promote biodiversity in mosaic landscapes, such as farmland (Smith et al., 2014) or forestry systems (Lindenmayer et al., 2006).

MULTIPLE-SCALE SPARING AND SOFTENING THE MATRIX

A multiple-scale land-sparing approach has the potential to benefit biodiversity beyond the specific spatial scales discussed above. In particular, sparing habitats at smaller spatial scales may benefit biodiversity at larger spatial scales by increasing resource availability for organisms that fundamentally depend on larger-scale habitat-patch networks. While conservation has traditionally focused on creating fairly large reserves on the basis, for example, of species-area relations or the aim of maximizing carrying capacity (e.g., Palomo et al., 2014), such approaches might not be practically feasible in heavily fragmented agricultural landscapes (Koh et al., 2009; Perfecto and Vandermeer, 2010). Thus, small-scale land sparing may increase the quality of the matrix surrounding biodiversity-rich patches and thereby deliver substantial biodiversity benefits (Ricketts, 2001; Fischer et al., 2006; Driscoll et al., 2013). A multiple-scale land sparing framework also has the potential of mitigating disruptions in symbiotic interactions in habitats of conservation concern by improving the quality of the surrounding landscapes (Pauw, 2007; Emer et al., 2013; Clough et al., 2014).

In Europe, semi-natural grasslands embedded in agricultural landscapes are highly species-rich and include many species of conservation concern (Steffan-Dewenter et al., 2014). Creating or managing field boundaries, woodlots or ponds, represent small-scale land-sparing options that might benefit grassland biodiversity across larger spatial scales by reducing the hostility of the landscape matrix (Donald and Evans, 2006). Thus, integrating conservation interventions in the form of a network of natural or semi-natural areas across agricultural landscapes (i.e., green infrastructure, see Maes et al., 2015) could benefit a range of species not primarily occurring in intensively managed farmland (Bergman et al., 2004; Öckinger and Smith, 2006, 2008).

However, while it has been argued that the integration of conservation strategies across landscapes is particularly important in the face of the dual threats of increasing land-use intensity and climate change (Hannah et al., 2002; Gillson et al., 2013), there are diverging views on how to achieve conservation goals. It has been suggested that improving habitats themselves, either quantitatively or qualitatively, offers a more effective conservation strategy compared with strategies that target the surrounding matrix (Hodgson et al., 2009, 2011). However, in particular in agricultural landscapes with fertile soils and hence high productivity, it might not be feasible to

expand source habitats because of high opportunity costs, in which case incentives targeting the matrix might be the only practical solution (Ekroos et al., 2014). Nevertheless, efforts to enhance the quality of the matrix for conservation are similarly constrained by high opportunity costs of any interventions that reduce yields. A combination of maintaining existing habitat fragments and creating small, high-quality grassland patches or resource-rich non-crop habitats between fields might, depending on landscape context, be the most effective strategy to promote biodiversity in highly fragmented agricultural landscapes (Baum et al., 2004; Rösch et al., 2013). The relative effectiveness of improving local habitat quality in fragments vs. improving matrix quality is likely to be highly context-dependent. Both perspectives offer hypotheses that can be tested in order to identify suitable strategies for specific conservation objectives.

MULTIPLE-SCALE SPARING AND ECOLOGICALLY SUSTAINABLE AGRICULTURE

In addition to food, feed and biofuels, agricultural systems play an important role in water and climate regulation, and the cycling and retention of nutrients (Norris et al., 2010). Agriculture is also ultimately dependent on a range of ecosystem processes that need to be accounted for in land-use decisions. Intensive agriculture that relies on high external inputs in terms of fertilizers and pesticides erodes ecosystem functions such as pollination, biological pest control, and nutrient cycling, which to a large extent rely on species commonly occurring in farmland (Tilman et al., 2002; Gaston and Fuller, 2008). So-called ecological intensification has been suggested as a means of increasing the sustainability of farming (Bommarco et al., 2013) by maintaining high yields through an increasing reliance on biodiversity-based ecosystem functions, or intermediate ecosystem services (Fisher et al., 2009). The challenge is to match the occurrence of beneficial biodiversity with the demand of the services provided by pollinating insects, predatory insects, or soil fauna, which in part depend on the spatial scale at which habitats are integrated across production lands (Mitchell et al., 2015). In the following we discuss how these demands can be met within the context of multiple-scale land sparing.

Important ecosystem service providers, such as pollinators and biological pest control agents, can be maintained by employing strategic interventions integrated within production lands (cf. Mitchell et al., 2015). Bees are central-place foragers that nest in various non-crop habitats and forage in local flower-rich habitats within their home-range. Because of their huge variety of life histories and ecological traits bee communities respond to land-use changes across multiple spatial scales. At local spatial scales, the disconnection between non-crop habitats, which provide nest sites for bees, and fields with insect-pollinated crops, can affect pollination services by wild bees and thereby crop yield. In contrast, pollination services provided by feral honey bees remain unaffected in such conditions (Garibaldi et al., 2011). Therefore, a land sparing strategy to promote wild pollinators should integrate non-crop habitat patches

evenly across production lands to ensure that insect-pollinated crops are located within the foraging range of pollinating insects. In addition, a greater proportion of natural habitats in the landscape can buffer wild bee communities from local impairments caused by pesticides (Park et al., 2015). Enhancing pollination services as such does not necessarily benefit rare species with specific habitat requirements (Kleijn et al., 2015). However, some important habitats for habitat specialists, such as semi-natural grasslands, also provide important nesting sites for pollinators, creating possible synergies between biodiversity conservation and ecosystem service provisioning (Macfadyen et al., 2012; Ekroos et al., 2014). The extent to which pollination in agricultural landscapes coincides with the occurrence of habitats targeting species of conservation concern is generally not known. Neither is it known whether there is a relationship between local habitat quality in semi-natural grasslands and pollination in the surrounding agricultural landscapes. With the loss of semi-natural grasslands the importance of sparing habitats at smaller spatial scales, such as non-crop field boundaries, increase, as they may provide substantial benefits for pollinating insects (Benjamin et al., 2014), although grassland habitat specialists are not likely to persist in such landscapes (Ekroos and Kuussaari, 2012).

In contrast to wild bees, generalist arthropod predators are not restricted to a local nest site and its surroundings. Instead, local assemblages of generalist predators are affected by the degree of disturbance of the local habitat (field), where land-use intensification represents increased disturbance and translates into less diverse predator communities (Rusch et al., 2014). Increasing non-crop habitat heterogeneity of the surrounding landscapes may alleviate this effect, where spared habitats act as source habitats through spill-over effects (Blitzer et al., 2012) or mass-effects (Leibold et al., 2004). A multiple-scale land-sparing approach will affect the landscape context surrounding local fields, which in turn can be managed with in-field interventions. To enhance biological pest control it becomes crucial to consider the interactions between in-field scale practices and larger scale changes of landscape composition for ecosystem services and service-providing units (Jonsson et al., 2014). Increasing landscape complexity by sparing non-crop habitats may, for example, affect parasitism rates of agricultural pests positively (Jonsson et al., 2012) or negatively (Menalled et al., 2003). This variability of the relationships between biological control and landscape complexity partly stems from variation in the effect of local farming practices on natural enemy and pest abundances (Rusch et al., 2010). It has therefore been suggested that in-field management interventions have the highest potential to promote biological control services in landscapes that provide little or no alternative non-crop habitats (Tscharntke et al., 2005).

In contrast to pollination services and biological pest control, the process of nutrient cycling cannot be segregated from production land in a meaningful way if it is to benefit arable production. The maintenance of soil fertility is essential for the long-term sustainability of agriculture, which demands management actions that enhance soil biodiversity at the level of individual fields (**Figure 1**). Nevertheless, the soil quality of arable land has been declining for decades in Europe, which

is worrying in terms of the sustainability of food production (Verheijen et al., 2009; JRC, 2012). Maintaining nutrient-rich top soils in agricultural land is important for increasing resistance to erosion, water-holding capacity, and soil fertility (Boardman, 2013). Because soil quality can only be managed at the local scale (Tscharntke et al., 2012b), a multiple-scale land-sparing approach cannot substitute in-field measures to decrease land-use intensity, such as using organic fertilizers, less intensive tillage practices, including legumes into crop rotations, or introducing rotational set-asides (Norris et al., 2010; Quinn et al., 2012).

PROSPECTS AND CONCLUSIONS

In this paper we have argued that land sparing should be seen as a strategy to be implemented simultaneously at multiple spatial scales. In transformed landscapes, only a multiple-scale approach can consider all the major ecological processes that determine levels of biodiversity, affecting habitat specialists and species of conservation concern across regions and common species essential for ecosystem functioning locally, on individual farms. The approach could be operationalized using a two-tiered approach: (1) on single farms, or amongst a group of collaborating farms, implement management strategies that increase, or maintain a heterogeneous landscape mosaic, and (2) on the basis of regional assessments of habitat availability and connectivity, create incentives for landowners to maintain specific habitats that are important for species of conservation concern (e.g., forests and forest remnants, semi-natural grasslands). Land-use decisions in landscapes dominated by farmland would, therefore, be made by farmers (guided by agri-environment schemes targeting key ecosystem processes) whereas biodiversity conservation should rely on identifying key landscape elements across larger spatial scales. Landscape elements identified as important for biodiversity conservation could thereafter be included as targeted measures within agri-environment schemes, which is currently the case e.g., in Sweden, regarding management of species-rich semi-natural pastures (Jordbruksverket, 2005). We believe that a multi-scale sparing approach offers the opportunity of combining biodiversity conservation with the ecological intensification of agriculture (Bommarco et al., 2013; Pywell et al., 2015), which will be needed if we are to produce increasing amounts of food and, simultaneously, decrease negative externalities of agriculture (Geiger et al., 2010; Vörösmarty et al., 2010; Rundlöf et al., 2015; Stehle and Schultz, 2015) while maintaining agricultural soil quality (Verheijen et al., 2009).

However, we also acknowledge that trade-offs may appear if investing resources in sparing habitats at one spatial scale reduces resources or opportunities for sparing habitat at other spatial scales. Given a fixed budget, trade-offs between e.g., buying land for a new nature reserve and financing small-scale habitat restoration in farmland under CAP obligations could potentially arise. Similarly trade-offs might arise between the goals of protecting biodiversity for its own sake vs. enhancing ecosystem service providers. For example, restoring habitats for grassland specialists may demand costly restoration interventions (Olsson and Ödman, 2014), whereas enhancing populations of ecosystem service providers could be effective with completely different

management interventions such as flower strips (Kleijn et al., 2015). The relative weighting of efforts at various spatial scales is a complicated issue that will depend on both the prioritization of different conservation goals and on the prevailing ecological conditions such as soil productivity, the strength of the links between service-providing organisms and yields (Ekroos et al., 2014), and whether synergies emerge between multiple-scale land-sparing interventions through e.g., source-sink dynamics between regions or habitats (Diffendorfer, 1998).

The amount of land that needs to be spared at various spatial scales remains an open question for future research. Across landscape mosaics only a few species persists when the proportion of semi-natural habitat is less than a few percentages (Tscharntke et al., 2005), although many of these are common species that are important providers of ecosystem services (Kleijn et al., 2015). There is little information on the landscape conditions under which ecosystem service provisioning varies over time (Bommarco et al., 2013; Fremier et al., 2013; Birkhofer et al., 2015), although we can expect a higher variability in ecosystem service flows over time in structurally simplified landscapes (Bengtsson et al., 2003; Tscharntke et al., 2012b). In addition, it is currently not well-known to what extent land sparing in landscape mosaics can replace in-field interventions, such as organic farming, to benefit local biodiversity (c.f. Gonthier et al., 2014).

There is not a single answer to the question of how much habitat needs to be spared at the level of habitat patch networks as species will differ strongly in their dispersal ability and tolerance of habitat degradation, including edge effects (Hanski, 2011, 2015). Reconciling biodiversity conservation and commodity production may therefore be particularly challenging at a regional scale in highly fragmented landscapes. On a longer time-scale, restoration of degraded land (Law and Wilson, 2015) or the rewilding of abandoned land (Navarro and Pereira, 2012; Ceașu et al., 2015) may provide new ways of increasing structural heterogeneity within landscape mosaics or habitat availability and level of connectivity across landscapes. Finally, at national or continental levels, it will be important to identify areas of particular importance for biodiversity to complement the selection of currently protected areas and minimize conflicts between agriculture and conservation. Shackelford et al. (2015) recently showed how such conflicts could be minimized on a global scale, with a focus on regions where agricultural expansion may take place at the expense of forest biodiversity, and where

increasing local land-use intensity (i.e., closing yield gaps) might threaten, for example, red-listed farmland or grassland species.

Trade-offs between biodiversity and agricultural production have spurred recent research on the relative benefits of land sparing and land sharing (Kremen, 2015). Given our definition of multiple-scale land sparing, the majority of studies seeking to reconcile biodiversity conservation with agricultural production have found land sparing to be the optimal solution (see e.g., von Wehrden et al., 2014). Irrespective of definitions, achieving efficient interventions and governance structures for land sparing at multiple scales will be challenging (Grau et al., 2013; Fischer et al., 2014). In Europe, designing interventions would call for close integration of small-scale land-sparing incentives which are currently governed by the EU Common Agricultural Policy, together with conservation efforts, such as the Natura 2000 network, implemented at larger spatial scales. Because agricultural production is becoming increasingly important on a global scale it will be crucial to identify the conservation strategies that return the greatest amount of biodiversity without compromising production (Phalan et al., 2011b). Using a multi-scale land sparing framework to solve trade-offs between biodiversity and production would ultimately demand that conservation strategies would be identified using e.g. efficiency frontiers optimizing production and levels of biodiversity at various spatial scales (Ekroos et al., 2014; Fischer et al., 2014; Butsic and Kuemmerle, 2015). Strategies are likely to be highly context-dependent as a consequence of regional variation in the types of habitat that are available for biodiversity and the soil fertility that determine the potential for arable production (Ekroos et al., 2014; Shackelford et al., 2015). We believe the optimal strategy will be a multi-scale strategy which, given multiple goals of conservation, can take very different forms depending on regional characteristics.

ACKNOWLEDGMENTS

This paper is based on a series of interdisciplinary workshops held in Lund during 2013 and 2014, dealing with the consequences of land sparing at various spatial scales. We thank Mark Brady and Yann Clough for valuable input during the workshops. The research was supported by the strategic research environment BECC, the strong research environment SAPES funded by FORMAS, and by the Era-net project MULTAGRI.

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Conflict of Interest Statement: 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.

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Soil Functional Zone Management: A Vehicle for Enhancing Production and Soil Ecosystem Services in Row-Crop Agroecosystems

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OPEN ACCESS

Edited by:

Karl Ritz,
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Spain

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 30 August 2015

Accepted: 14 January 2016

Published: 05 February 2016

Citation:

Williams A, Kane DA, Ewing PM, Atwood LW, Jilling A, Li M, Lou Y, Davis AS, Grandy AS, Huerd SC, Hunter MC, Koide RT, Mortensen DA, Smith RG, Snapp SS, Spokas KA, Yannarell AC and Jordan NR (2016) Soil Functional Zone Management: A Vehicle for Enhancing Production and Soil Ecosystem Services in Row-Crop Agroecosystems. *Front. Plant Sci.* 7:65. doi: 10.3389/fpls.2016.00065

There is increasing global demand for food, bioenergy feedstocks and a wide variety of bio-based products. In response, agriculture has advanced production, but is increasingly depleting soil regulating and supporting ecosystem services. New production systems have emerged, such as no-tillage, that can enhance soil services but may limit yields. Moving forward, agricultural systems must reduce trade-offs between production and soil services. Soil functional zone management (SFZM) is a novel strategy for developing sustainable production systems that attempts to integrate the benefits of conventional, intensive agriculture, and no-tillage. SFZM creates distinct functional zones within crop row and inter-row spaces. By incorporating decimeter-scale spatial and temporal heterogeneity, SFZM attempts to foster greater soil biodiversity and integrate complementary soil processes at the sub-field level. Such integration maximizes soil services by creating zones of ‘active turnover’, optimized for crop growth and yield (provisioning services); and adjacent zones of ‘soil building’, that promote soil structure development, carbon storage, and moisture regulation (regulating and supporting services). These zones allow SFZM to secure existing agricultural productivity while avoiding or minimizing trade-offs with soil ecosystem services. Moreover, the specific properties of SFZM may enable sustainable increases in provisioning services via temporal intensification (expanding the portion of the year during which harvestable crops are grown). We present a conceptual model of ‘virtuous cycles’, illustrating how increases in crop yields within SFZM systems could create self-reinforcing feedback processes with desirable effects, including mitigation of trade-offs between yield maximization and soil ecosystem services. Through the creation of functionally distinct but interacting zones, SFZM may provide a vehicle for optimizing the delivery of multiple goods and services in agricultural systems, allowing sustainable temporal intensification while protecting and enhancing soil functioning.

Keywords: crop yield, ecosystem services, precision tillage, soil biodiversity, soil management, temporal intensification, trade-offs, zonal tillage

INTRODUCTION

Intensification of agriculture has been vital for increasing global food supply and alleviating hunger for millions of people (Godfray et al., 2010). In addition, intensification is key to meeting growing demand for bioenergy feedstocks and a wide variety of bio-based products (Jordan et al., 2007; McCormick and Kautto, 2013). However, agricultural intensification has also resulted in damage to the environment. In particular, soils in many regions of the world have been degraded by intensive agricultural practices (Mäder et al., 2002; Tilman et al., 2002; Heenan et al., 2004), and this has led to increased societal demand for more sustainable agricultural production systems (Foley et al., 2011; Kremen and Miles, 2012). In response, new management strategies have emerged, including soil-focused approaches such as no-tillage, which aim to improve soil regulating and supporting ecosystem services by reducing soil disturbance (Hobbs et al., 2008; Baveye et al., 2011; Palm et al., 2014). However, no-tillage often results in reduced yields (Giller et al., 2009; Pittelkow et al., 2015), highlighting trade-offs between soil and provisioning services. Such trade-offs are highly problematic, given that global demand for food and other agricultural products is expected to rise considerably by 2050 (Godfray et al., 2010; Tilman et al., 2011). Furthermore, to limit the need to convert additional lands to agriculture (i.e., extensification), the world's existing crop production systems must become more productive (Foley et al., 2011; Bommarco et al., 2013; Godfray and Garnett, 2014).

One option for securing the productivity of existing agricultural land while also enhancing delivery of soil ecosystem services is by integrating the high productivity of intensive field crop production systems (including intensive tillage) with the improvements in soil quality associated with stringent limitations on tillage. Herein, we present evidence that a novel approach to management of field crop agroecosystems – soil functional zone management (SFZM) – can promote such integration. As detailed below, SFZM entails the creation and management of distinct yet complementary soil functional zones that have potential to reduce trade-offs between short-term productivity and soil quality.

We believe SFZM to be a previously unrecognized strategy for expanding the range of ecosystem service production from field crop agroecosystems. Several forms of SFZM (e.g., ridge tillage and strip tillage) have been studied extensively in terms of their effects on a range of crop and soil attributes. Here, we expand upon this level of analysis and understanding through a broad exploration of ecosystem service production and underlying agroecological processes in SFZM, drawing on a wide range of evidence and identifying critical knowledge gaps in understanding of SFZM. In our analysis, we focus first on supporting and regulating services, and then examine the potential of SFZM to increase productivity of agricultural systems (i.e., enhance provisioning services). In particular, we consider the role of SFZM in supporting temporal intensification, which aims to enhance provisioning services by expanding the annual time period in which harvestable crops are grown. We consider the potential dynamics of agroecosystems under SFZM, and the role of these dynamics in improving

the sustainability of temporal intensification. We focus on the dynamic implications of ‘virtuous cycles’ (self-reinforcing feedback processes with desirable effects) that may occur in SFZM. Such feedback processes may serve to reduce trade-offs between provisioning, supporting, and regulating services in temporal intensification.

SOIL FUNCTIONAL ZONE MANAGEMENT

Soil functional zone management is a novel concept of field crop agroecosystem management that seeks to create distinct, yet functionally complementary soil zones through non-uniform management of tillage and crop residues. These zones can be tailored for a variety of different functions or ecosystem services and can be permanent or change locations between seasons. At its most basic, SFZM involves a zone of ‘active turnover’, managed to optimize conditions for seed germination and crop growth; and an adjacent ‘soil building’ zone, which is managed to protect soil organic matter (SOM), enhance soil water holding capacity and provide habitats for soil organisms. At present, the two most widely practiced implementations of SFZM are ridge tillage and strip tillage (**Figure 1**). While SFZM does not necessarily involve novel management practices (e.g., ridge tillage has been practiced since the 1980s), it provides a novel framework for enhancing ecosystem service production in field crop agroecosystems.

Soil functional zone management differs markedly from conventional and no-tillage practices, which can both be characterized as non-zonal, or uniform. For example, in a chisel plow system, topsoil and crop residues are uniformly mixed, creating a relatively homogenous soil environment across a tilled field (Mannering and Fenster, 1983). In no-tillage, the soil is left undisturbed and crop residues are retained, providing uniform residue cover on the soil surface (Mannering and Fenster, 1983; Hobbs et al., 2008; **Figure 1**). Despite advances in precision agricultural application of fertilizer and agrochemicals, tillage is still predominantly applied homogeneously (Lal, 2015).

Through creation and management of differentiated soil zones (**Figure 1**), SFZM creates spatial heterogeneity over small (<1 m) spatial scales. Relative to non-zonal tillage, such enhancement of within-field heterogeneity across space and time serves to enhance the range of soil physical conditions and functional biodiversity within a row-crop agroecosystem. Increasing heterogeneity can enhance biodiversity by providing habitat and other key resources to a wider range of organisms. This expansion of resource diversity in space and time can support effective resource partitioning and increased diversity of microhabitats, allowing coexistence of soil organisms and increased functional biodiversity (Ettema and Wardle, 2002; Kremen and Miles, 2012). In turn, increased functional biodiversity can support provisioning services while simultaneously conserving or enhancing a range of soil services, including organic matter decomposition and nutrient turnover, soil carbon storage, and pathogen suppression (Coleman et al., 2004; Birkhofer

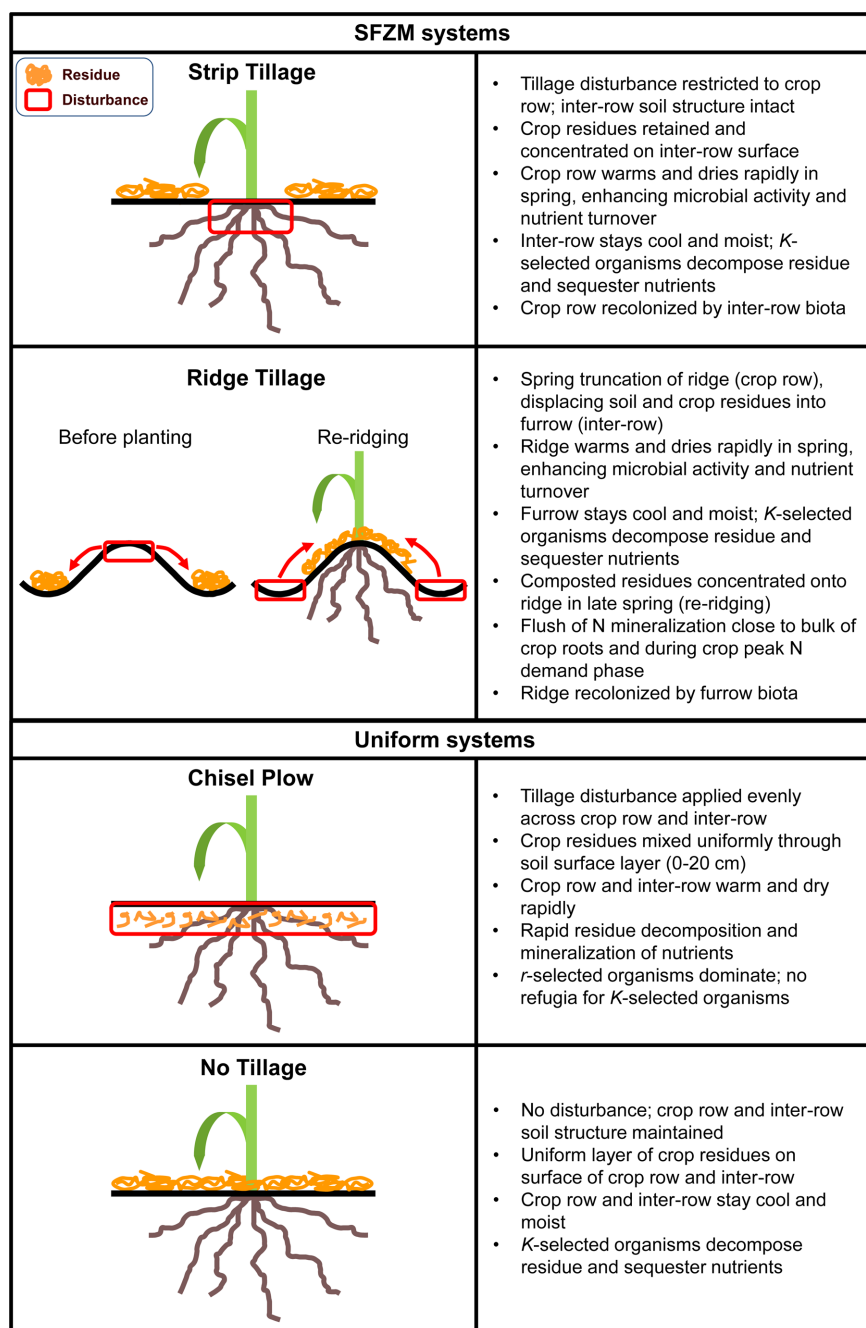


FIGURE 1 | Examples of typical soil functional zone management (SFZM) and uniform tillage systems.

et al., 2008; van der Heijden et al., 2008; Suzuki et al., 2013).

Conventional soil management is typically characterized by frequent and intense disturbance (e.g., tillage and agrochemicals) combined with low plant resource diversity (e.g., monocultures and minimal crop residue). These factors lead to reduced abundances and diversity of soil organisms in conventional systems compared with no-tillage and other systems with reduced tillage and more diverse crop rotations (Wardle, 1995; Kabir,

2005; Culman et al., 2010; Postma-Blaauw et al., 2010). Moreover, these factors selectively alter soil biotic communities, leading to dominance by *r*-selected organisms (organisms adapted for rapid reproduction and dispersal; Pianka, 1970; Verbruggen and Kiers, 2010). For example, larger-bodied soil organisms are reduced in abundance relative to smaller-bodied organisms, leading to reductions in faunal and fungal biomass, and shifts toward bacterial dominance (de Vries et al., 2006; Postma-Blaauw et al., 2010). The adoption of no-tillage management has been

demonstrated to improve the abundance and diversity of soil communities, such that they more closely resemble undisturbed grasslands (Postma-Blaauw et al., 2012; S  le et al., 2015). SFZM entails limited and targeted disturbance across both space and time, and maintenance of crop residues, thereby providing undisturbed or minimally disturbed soil refugia (**Figure 1**). We hypothesize that these refugia can support faunal and fungal diversity in a similar way to no-tillage, and provide a base from which slow-growing organisms with longer generation times (*K*-selected organisms) might be able to recolonize disturbed areas. In essence, we propose that SFZM, by expanding both habitat and resources relative to conventional soil management, can enhance both provisioning and soil regulating and supporting services by enhancing soil biodiversity.

SFZM AND SOIL ECOSYSTEM SERVICES

Securing high levels of agricultural production while simultaneously improving regulating and supporting soil ecosystem services requires management strategies that expand the range of service production (Foley et al., 2011; Bommarco et al., 2013). As outlined above, by providing spatial and temporal heterogeneity in terms of tillage and crop residue distribution, we hypothesize that SFZM is one such strategy. In the following sections, we present and examine evidence that SFZM can, in fact, enhance soil ecosystem service delivery.

Supporting Soil Services

Services Produced by Soil Biota

The creation of undisturbed refugia for soil microbiota, particularly filamentous fungi, through targeted disturbance is one pathway by which SFZM may increase the supply of supporting soil services. Such refugia should impact carbon (C), nitrogen (N), and phosphorus (P) cycling to the benefit of above-ground productivity. Nutrient cycling among organic and inorganic pools is driven by microbial turnover, with fungi generally thought to be more effective at storing C and N in organic matter than bacteria (Six et al., 2006), while the higher turnover rate of bacteria promotes gross mineralization and plant nutrient uptake (Schimel and Bennett, 2004). Filamentous, saprophytic fungi are also the dominant decomposers of recalcitrant plant litter, producing more degradative enzymes than bacteria (Treseder and Lennon, 2015). Arbuscular mycorrhizal fungi (AMF), meanwhile, are well-known to dominate plant P nutrition, and their central role in C and N cycling is increasingly recognized (Hodge and Storer, 2015). Thus, a combination of bacteria- and fungi-rich communities is desirable for efficient nutrient cycling.

Soil communities under conventional tillage generally have altered structural, morphological, and functional profiles compared to communities under no-tillage. Overall, tillage lowers microbial biomass, enzyme activities, and nutrient cycling rates (Kladivko, 2001; Balota et al., 2014). While tillage does not necessarily alter fungal:bacterial ratios directly, as bacterial biomass also tends to decrease with tillage (Strickland and

Rousk, 2010), lower levels of soil moisture under conventional tillage do reduce fungal:bacterial ratios (Frey et al., 1999). As well, tillage reduces AMF community diversity, creating lower diversity subsets of no-tillage communities (Verbruggen et al., 2012). Those AMF that remain are *r*-selected, producing more reproductive spores and fewer soil-exploring hyphae than *K*-selected AMF (Verbruggen and Kiers, 2010). The *r*-selected AMF recover quickly from disturbance but are less efficient at delivering resources to crops (Powell et al., 2009; Verbruggen and Kiers, 2010).

Under SFZM, both disturbed and undisturbed regions are directly adjacent to each other (**Figure 1**). The disturbed region exposes labile organic matter and aerates the soil, providing excellent conditions for nutrient turnover immediately after disturbance (Martens, 2001), while the undisturbed region creates a refuge for slower-growing, more sensitive filamentous fungi and hyphae-intensive AMF. From this refuge, these organisms can quickly re-colonize the mixed and aerated disturbed region. The 'refuge and recolonization' process may enhance organic matter production and nutrient cycling. Slow-growing *K*-selected fungi contribute to long-term organic matter pools through necromass production and through the formation of protective soil aggregates (Six et al., 2006; Crowther et al., 2015; Ludwig et al., 2015). As primary decomposers of crop residues, they also have unique ability to access N-rich soil and C-rich crop residues simultaneously, transporting C from residue to soil, and N from soil to residue (Hendrix et al., 1986; Frey et al., 2000, 2003). Tillage disrupts the hyphal networks of these fungi, thereby limiting the production of these services. However, disturbance does enhance residue-soil contact to speed colonization by decomposers. Therefore, the creation of two functionally distinct, adjacent zones under SFZM – an undisturbed fungal refuge and an area where residue is mixed well with soil – should facilitate decomposition of crop residue and the formation of organic matter.

Such refugia may explain enhanced P delivery to maize (*Zea mays* L.) by AMF in SFZM systems (McGonigle and Miller, 1993). P-limitation is a common problem for cereal production in many temperate growing regions, especially on calcareous, P-fixing soils (Holloway et al., 2001). In such a region, young maize plants were found to accumulate greater quantities of P under SFZM (ridge tillage) than under uniform tillage (chisel plow), which was due to greater mycorrhizal activity in the ridge (McGonigle et al., 1990; McGonigle and Miller, 1993, 1996). Based on more recent studies of mycorrhizal P delivery to a variety of plant species, increased P delivery may result from increases in the abundance of Diversisporaceae (formerly Gigasporaceae). This family of AMF develops more extensive soil hyphae and is more effective at delivering P to host plants than other AMF families (Glomeraceae and Acaulosporaceae; Powell et al., 2009). Tillage strongly hinders Diversisporaceae activity (Verbruggen and Kiers, 2010), but the targeted disturbance of ridge top removal and later reformation (**Figure 1**) likely enables them to persist in ridge tillage systems (Ewing et al., unpublished).

In addition to fungi and bacteria, soil fauna may be better protected in SFZM systems. Soil fauna contribute to important agroecosystem services, including decomposition,

nutrient cycling, bioturbation, and pest suppression (Coleman et al., 2004; Birkhofer et al., 2008; Suzuki et al., 2013). For example, soil macrofauna facilitate decomposition by fragmenting and redistributing plant residues in the soil profile (Brussaard et al., 2007; García-Palacios et al., 2013). It is well-established that tillage acts as a strong physical filter on soil faunal communities (Roger-Estrade et al., 2010). In a vegetable production system, the combination of reduced tillage (active turnover zone) and no-tillage (soil building zone) in SFZM strip tillage systems maintained higher earthworm and nematode populations compared to conventional, uniform tillage systems (Overstreet et al., 2010). Furthermore, when strip tillage was combined with strategic management of cover crop residues, predatory mite and collembolan (fungivore) densities and nematode community complexity increased compared to conventionally managed systems (Wang et al., 2011).

Nitrogen Cycling

Soil functional zone management systems may also enhance crop N nutrition by promoting greater synchrony between soil N availability and crop N requirements. Crop N demand varies over the growing season, and is greatest for row crops during vegetative growth (Olson and Kurtz, 1982; Robertson, 1997), which generally happens in mid- to late- summer. When fertilizer N is supplied at the time of planting, the resulting asynchrony with crop demand can encourage weed growth, lead to inefficient crop use of fertilizer, and drive N loss from soils via denitrification or leaching (Robertson, 1997; Crews and Peoples, 2005; Shanahan et al., 2008). These problems can be addressed by management that synchronizes N supply with peak crop N demand.

The key to N synchrony may be to manage N supply in both space and time (Shanahan et al., 2008). This is a central feature of SFZM, especially when redistribution of plant residues into the crop row is involved, such as under ridge tillage (John et al., 2004). Under a range of row crops and crop rotations, ridge tillage creates higher concentrations of soil organic C (SOC; Shi et al., 2012), potentially mineralizable N, microbial N (Müller et al., 2009b) and microbial biomass (Bezdicsek et al., 2003; Grigera et al., 2007; Müller et al., 2009b) on the ridge-tops of crop rows compared with inter-rows. This spatial concentration of resources and microbial biomass leads to increased microbial activity in the crop row (Clay et al., 1995; Liebig et al., 1995; Müller et al., 2009a), and increases rates of N mineralization (Figure 2; Kane et al., 2015). Thus, ridge tillage appears to synchronize potentially mineralizable N supply with crop demand in both space and time, resulting in greater crop N uptake (Gordon et al., 1993; Kane et al., 2015). Similar increases in N mineralization have been observed in the inter-row spaces of strip tillage systems of both maize and orange trees (*Citrus sinensis* L.) Osbeck; Johnstone et al., 2009; Balota and Auler, 2011), but strip tillage was not found to improve N synchrony in a cabbage (*Brassica oleracea* L.) system (Haramoto and Brainard, 2012). This may indicate that the redistribution of plant and soil residues that occurs during ridge tillage is the key to unlocking the N synchrony potential of SFZM. Furthermore, to the extent that SFZM encourages nutrient recycling ecosystem services, then synchronized N can be supplied from internal sources (crop

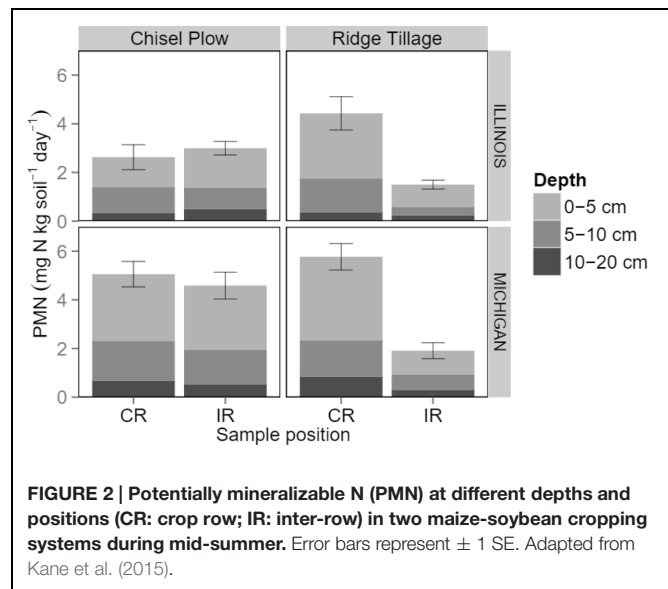


FIGURE 2 | Potentially mineralizable N (PMN) at different depths and positions (CR: crop row; IR: inter-row) in two maize-soybean cropping systems during mid-summer. Error bars represent ± 1 SE. Adapted from Kane et al. (2015).

residue, cover crop, or weed residues), reducing the need for fertilizer inputs.

Potential Trade-Offs

Despite the wide range of benefits that may result from SFZM, undesirable effects may also arise, creating trade-offs associated with SFZM. Undesirable effects include the potential for increased populations of some pests due to less frequent and less intense tillage operations (Chaplin-Kramer et al., 2011). For example, incidence of *Rhizoctonia* root rot and parasitic nematodes increased in no-tillage systems with residue retention compared with conventional tillage (Schroeder and Paulitz, 2006; Govaerts et al., 2007). However, when used in combination with other pest management practices, like diverse crop rotations, SFZM strategies that include an intra-seasonal tillage event, such as ridge tillage, can help disrupt pest populations while maintaining natural enemy populations (McKeown et al., 1998). Pruess et al. (1968) observed clustering of western corn rootworm (*Diabrotica virgifera* Le Conte) eggs in furrow positions and delayed larval development following an intra-seasonal ridging event. They suggested the ridging event relocated the previously uniformly dispersed eggs into the furrow while also burying the eggs under surface debris, lowering soil temperatures, and slowing larval development (Pruess et al., 1968). Additional research on the effects of timing of intra-seasonal tillage on pest and natural enemy populations will be necessary to further minimize pest management trade-offs associated with SFZM.

Regulating Soil Services

Soil Structure, Moisture, and Carbon Storage

The accumulation of SOM in agricultural systems has important implications for soil structure development (Bronick and Lal, 2005; Lal, 2009). SOM is a primary building block of aggregates – it serves to bind and stabilize soil micro-aggregates, which in turn coalesce to form macro-aggregates (Tisdall and Oades, 1982; Bronick and Lal, 2005; Lützow et al., 2006; Karami et al.,

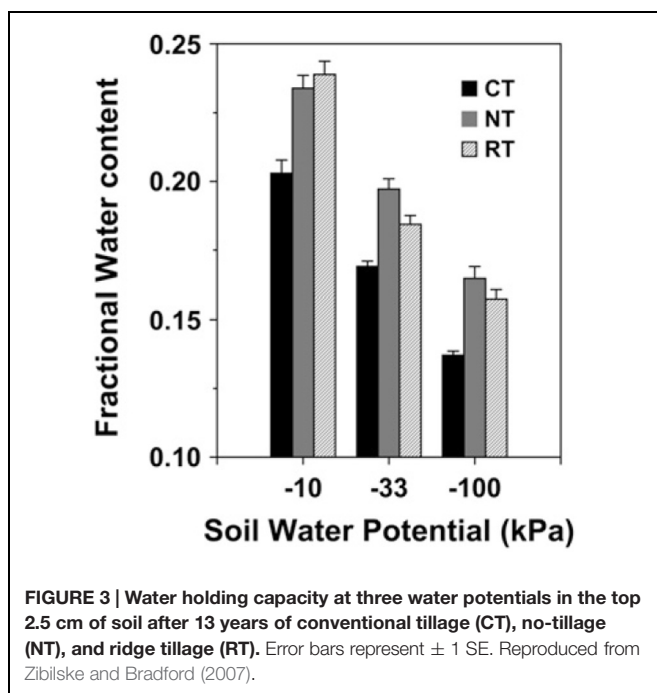
2012). Soil tillage and residue management affect aggregate development through their collective influence on SOM quality and accrual. Previous studies have found that SFZM systems increase organic matter (OM) in surface soil layers (0–15 cm) relative to conventional tillage (Angers et al., 1995; Unger, 1995). In turn, SFZM systems, much like no-tillage systems, have been found to increase aggregate stability and average size relative to conventional tillage (Kladivko et al., 1986; Mikha and Rice, 2004; Zibilske and Bradford, 2007). The relative improvements to soil structure in these studies were attributed to minimal tillage-induced disturbance to larger, more fragile aggregates.

The physical encapsulation of OM within soil aggregates plays an important role in the accumulation of soil C (Balesdent et al., 2000; Grandy and Robertson, 2007; Plaza et al., 2013). The OM contained within macro-aggregates is labile and particulate in nature, while micro-aggregate C is more stable, having undergone microbial processing (Elliot, 1986; Plaza et al., 2013; Zhang et al., 2013). Macro-aggregates are highly sensitive to management, with their stability depending largely on plant roots, fungal hyphae, tillage intensity, and microbial activity (Six et al., 2000; Rillig and Mummey, 2006; Zhang et al., 2013). In conventional systems, where macro-aggregate structures are regularly broken down, labile forms of C are released from physical protection resulting in rapid SOM depletion (Grandy and Robertson, 2006, 2007; Panettieri et al., 2015). The reduction in soil disturbance under SFZM increases soil aggregate formation, and the process of concentrating crop residues in inter-row positions has been found to increase concentrations of SOM (Unger, 1995).

The improvement of soil structure via enhanced aggregate formation under SFZM provides regulating services by facilitating rainfall infiltration and enhancing soil water holding capacity (Figure 3; Franzluebbers, 2002; Zibilske and Bradford,

2007). SFZM systems have been shown to conserve soil moisture more effectively than conventional tillage systems (Drury et al., 2006; Zibilske and Bradford, 2007; Williams et al., under review). This feature may be particularly important in terms of adapting agricultural systems to drought stress. Droughts are predicted to increase in frequency and severity with climate change (Gornall et al., 2010; Trenberth et al., 2014). No-tillage has been highlighted as a drought management option due to its ability to conserve soil moisture (Lal, 2004; Powlson et al., 2014). SFZM, because it features zones of no or reduced tillage, may therefore play a crucial role in buffering agricultural systems against drought, while minimizing trade-offs with provisioning services associated with no-tillage (Pittelkow et al., 2015). Put another way, SFZM may help build resilience to climate change while protecting long-term agricultural productivity.

In addition, we hypothesize that the heterogeneous soil environments created by SFZM allow development of greater fungal biomass by providing refugia from tillage disturbance (see Services Produced by Soil Biota above); fungal hyphae play an important role in the formation and stability of soil aggregates (Wilson et al., 2009; Peng et al., 2013; Lehmann and Rillig, 2015). Recent studies lend support to this hypothesis, as reduced tillage systems have been shown to promote greater fungal biomass and diversity relative to conventional tillage systems (van Groenigen et al., 2010; S  le et al., 2015). Furthermore, crops grown under ridge tillage have shown greater mycorrhizal colonization compared with crops grown under uniform tillage systems (McGonigle and Miller, 1993; McGonigle et al., 1999). Thus, by providing greater long-term protection of SOC by enhancing aggregate formation, SFZM could potentially reduce the release of CO₂ and other greenhouse gasses back to the atmosphere, thereby helping to mitigate the contribution of agriculture to climate change.



Additional Regulating Services: The Case of Weed Control

Soil functional zone management may also provide regulating services that contribute to the suppression of weeds. Non-herbicidal weed suppression services will become increasingly valuable as populations of weeds that are resistant to glyphosate and other herbicides continue to become more abundant. The problem of herbicide resistant weeds is especially acute in conventional no-tillage systems, and particularly in those systems that rely on herbicide resistant crops, because of their exclusive reliance on herbicides for weed control (Mortensen et al., 2012). SFZM, through a variety of mechanisms, may reduce weed density and growth, shift the competitive balance from weeds to crops, and provide more opportunities for integrated weed management than conventional no-tillage or other uniformly managed systems.

One way that SFZM can contribute to the management of weeds is through promotion of AMF. AMF can suppress the development of both AMF host and non-host weed species (Jordan et al., 2000; Vatovec et al., 2005), thereby reducing crop yield losses to weeds (Rinaudo et al., 2010; Veiga et al., 2011). Several studies have found negative correlations between AM colonization and crop growth in no-tillage systems relative

to conventional tillage, which has been attributed to cooler temperatures in no-tillage crop rows as a result of residue cover (McGonigle and Miller, 1996; McGonigle et al., 1999). SFZM may overcome such drawbacks by removing crop residues from crop rows and concentrating them in relatively undisturbed inter-rows (**Figure 1**). This uncoupling of soil temperatures and residues from areas of soil disturbance allows soil in row positions to warm more rapidly early in spring, while preserving an extensive AMF mycelial network for rapid root colonization in inter-rows (Johnson et al., 1997). Maize grown under ridge tillage has been shown to have increased mycorrhizal colonization and enhanced early season crop performance relative to no-tillage (Vivekanandan and Fixen, 1991; McGonigle and Miller, 1993). When AMF colonize multiple hosts they can increase nutrient transfer to the host that provides the most carbohydrates (Lekberg et al., 2010; Kiers et al., 2011). As such, by improving crop establishment and vigor relative to no-tillage, SFZM can alter interactions between crops and weeds via AMF, improving crop nutrition and performance, and inhibiting weed development. Such improvements have been demonstrated in a strip tillage system, where tomato (*Solanum lycopersicum* L.) performance was improved by AMF when in competition with bahiagrass (*Paspalum notatum* Flüggé; Sylvia et al., 2001). However, further research is needed to quantify the contribution of AMF to weed suppression in addition to crop performance within SFZM systems.

Soil functional zone management may enhance weed suppression in other ways, particularly when integrated with cover crops. Cover crops present in inter-rows can suppress weeds through resource and light competition (Liebman and Dyck, 1993; Teasdale, 1996), disruption of weed life cycles (Moyer et al., 2000), physical suppression by cover crop residues (Moore et al., 1994), and release of phytotoxic chemicals (Kruidhof et al., 2009; Teasdale et al., 2012; Samedani et al., 2013). Release of phytotoxic chemicals from cover crop residues can also have negative effects on crop species (Kruidhof et al., 2011; Soltys et al., 2012), and this can be particularly true in uniform tillage systems. SFZM, particularly in ridge tillage systems, removes residues from the crop row and concentrates them in inter-row positions (Hatfield et al., 1998; **Figure 1**). Therefore, by actively managing the placement of phytotoxic cover crop residues, SFZM can minimize some of the potential trade-offs associated with the use of cover crops. The process of concentrating crop residues also promotes survival of soil pathogens in inter-row positions, by increasing inter-row soil moisture content (Cook and Haglund, 1991; Page et al., 2013; Manstretta and Rossi, 2015); weed seeds on or near the soil surface in inter-row positions are then subject to pathogen attack (Caesar, 2005), while crop seeds in the row avoid such attack.

The concentration of crop residues in inter-rows under SFZM may further control weeds by smothering and reducing light penetration to the soil, reducing weed emergence (Forcella and Lindstrom, 1988; Kruidhof et al., 2009). The re-ridging event in ridge tillage, where residues and soil are moved from the inter-row and concentrated on ridges (**Figure 1**), can also serve to smother weeds growing in the crop row (Buhler, 1992). The combination of concentrated crop residues and reduced thermal

time accumulation in SFZM systems may provide an additional weed control mechanism.

SFZM AND PROVISIONING SERVICES

In our presentation of SFZM hitherto, we have sought to establish that improvements in soil regulating and supporting services can be achieved while maintaining existing levels of agricultural output. The successful integration of conventional, intensive agricultural management approaches with more environmentally sustainable practices such as no-tillage would represent a major advance in agronomy. However, given expected increases in global demand for food and other agricultural products by 2050 (Godfray et al., 2010; Tilman et al., 2011), and the need to limit conversion of additional lands to agriculture, it is not sufficient for the world's existing crop production systems to maintain current levels of production; they must become more productive (Foley et al., 2011; Bommarco et al., 2013; Godfray and Garnett, 2014).

Temporal Intensification

One way of increasing the productivity of existing agricultural land is through temporal intensification, which aims to expand the annual time period in which harvestable crops are grown. Practices aimed at temporally intensifying agriculture are being increasingly implemented around the world (Ray and Foley, 2013). These include increasing crop harvest frequency per unit area and time by double or triple cropping (Heaton et al., 2013; Ray and Foley, 2013), and earlier planting of cultivars with longer maturation times (Sacks and Kucharik, 2011).

Temporal intensification may improve soil services by reducing or eliminating periods when soil is left bare or fallow. By replacing bare-soil fallows with live plant communities during some or all of the year, temporal intensification can provide a range of soil related regulating and supporting services, such as reduced rates of soil erosion and nutrient leaching (Dabney et al., 2001; Dean and Weil, 2009), increased microbial community size and activity (McDaniel et al., 2014; Tiemann et al., 2015), and weed suppression (Davis and Liebman, 2003; Carrera et al., 2004). In addition, temporal intensification provides opportunities to increase crop rotational diversity (Moore and Karlen, 2013). These factors enhance crop residue, root and exudate production, providing increased C resources for microbial processing (Kong et al., 2011; Tiemann et al., 2015), with subsequent soil quality benefits including long-term C storage and improved soil structure (Grandy and Robertson, 2007; Schmidt et al., 2011; Poeplau and Don, 2015; Tiemann et al., 2015).

Despite the potential benefits of temporal intensification, there are also large potential drawbacks, including reductions in the yields of each crop when multi-cropping is used for temporal intensification (Tonitto et al., 2006; Johnson et al., 2015). Such reductions may be severe if soil resources are exhausted or tied up by previous crops or their residues, or if harvest of one crop delays planting of the next crop. Such delays and the lack of operational flexibility they incur can severely limit production capacity. Other potential drawbacks include damage to soil

structure from increases in soil cultivation intensity (Grandy and Robertson, 2006), and greater nutrient leaching and depletion of water resources due to increased fertilization and irrigation (Ju et al., 2009; Ray and Foley, 2013). Soil biodiversity may also be reduced by temporal intensification due to the deleterious effects of increased soil cultivation and elevated input of agrochemicals (Helgason et al., 1998; Mäder et al., 2002). Loss of soil biodiversity may curtail ecosystem functions that generate soil ecosystem services (Bardgett, 2005).

To mitigate these potential downsides while still realizing the inherent benefits of temporal intensification, novel management systems are needed. These systems must enable increases in the amount of product that can be extracted over a given time period while simultaneously protecting soil functional biodiversity and building soil quality. We contend that SFZM is a particularly promising strategy for achieving sustainable temporal intensification because it involves the creation of functionally distinct yet complementary soil zones. Through the integration of conventional, intensive management and reduced tillage practices, these zones are optimized for crop productivity (active turnover zone) and soil protection (soil building zone).

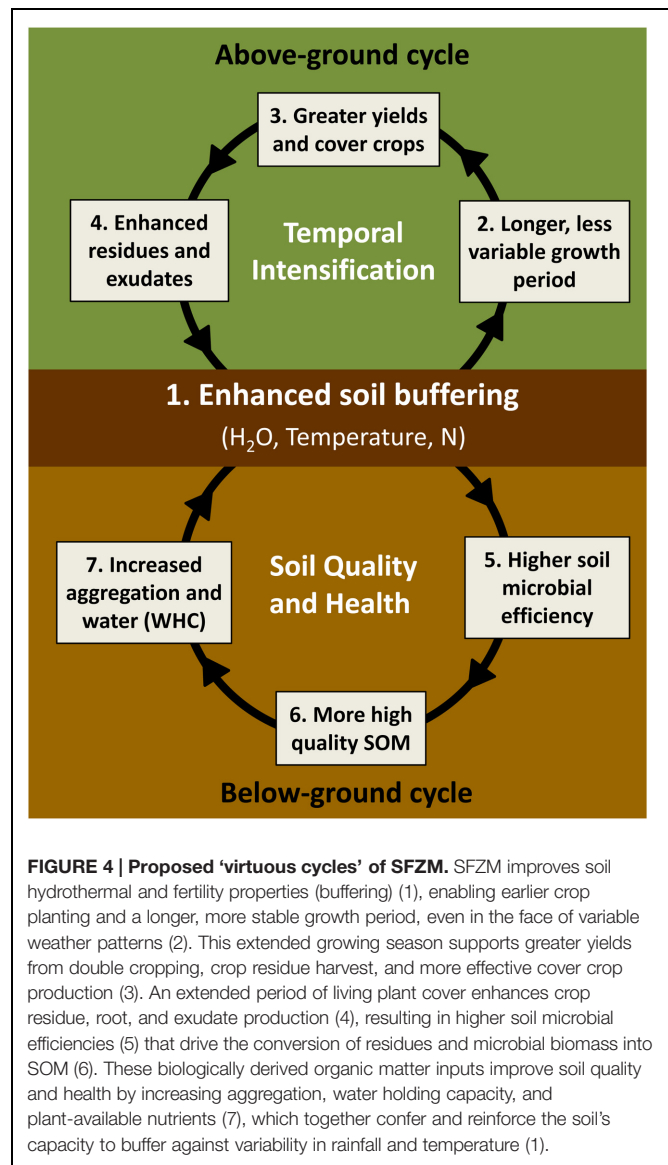
Dynamics of SFZM: Potential for a Virtuous Cycle Linking Yield and Soil Quality

We base our hypothesis of joint enhancement in provisioning and other ecosystem services via SFZM on a virtuous cycle model that links above-ground and below-ground processes (Figure 4). Specifically, we propose that SFZM engenders a self-reinforcing feedback process that couples improvements in soil regulating and supporting services (below-ground cycle) with improvements in provisioning services via increased field working days (above-ground cycle).

Above-Ground Processes in the Virtuous Cycle

A key component of sustainable temporal intensification is increasing the period of time during which crops can be grown and harvested on existing agricultural land. In real terms, this translates into a need for increased field working days, which can be achieved by enabling earlier soil cultivation and planting, by supporting crop growth later in the season, or by a combination of both.

Existing SFZM systems (e.g., ridge and strip tillage), which remove crop residues from crop row positions prior to planting, have been demonstrated to produce seedbed environments that warm and dry rapidly in early spring (Hatfield et al., 1998; Licht and Al-Kaisi, 2005). These seedbeds have similar hydrothermal properties to conventional tillage systems, which in turn have improved hydrothermal properties relative to no-tillage systems, i.e., are warmer and drier, resulting in improved seedling emergence relative to no-tillage (Cox et al., 1990; Kovar et al., 1992; Dwyer et al., 2000). Planting date has a large influence on crop productivity, and delays in planting due to climate fluctuations can severely reduce yields (Deryng et al., 2014). On poorly drained, finely textured soils, or during periods of excessive rainfall, ridge tillage can also improve seedbed



hydrothermal conditions above that of conventional tillage, leading to earlier planting, greater accumulation of thermal time and improved yields (Cox et al., 1990; Eckert, 1990; Fausey, 1990). This provides the basis for an important premise of the virtuous cycle model (Figure 4): that SFZM increases field working days by allowing cultivation and planting to occur earlier in the season compared to when these operations could occur, for example, in an adjacent field managed with no-tillage approaches. SFZM would also likely outperform conventional tillage in terms of field working days in poorly drained soils or in years with wet springs (Figure 4, points 1 and 2).

Soil functional zone management can also extend the growing season by continuing to support crop growth later in the season. Existing SFZM systems concentrate soil moisture into crop inter-row positions (Müller et al., 2009b; Shi et al., 2012), substantially increasing soil moisture above that of conventional systems and maintaining it at levels similar or equivalent to no-tillage (Drury

et al., 2003, 2006). These moisture-rich inter-rows may provide an important water resource during critical periods of crop development (Alvarez and Steinbach, 2009). Thus, by altering soil hydrothermal properties, SFZM can increase field working days at both ends of the growing season; allowing soil to be cultivated and/or planted earlier in the season, and maintaining soil moisture in inter-rows that can sustain crop growth later in the season or support planting of winter double crops. In other words, SFZM creates functionally distinct zones that together provide greater soil buffering to climate variability; SFZM buffers against extremes in soil temperature and moisture, and thereby provides a longer, less variable growth period (**Figure 4**, points 1 and 2).

The extension of the growing season afforded by SFZM enables greater utilization of solar radiation both at the beginning and end of the growing season, particularly in northern temperate regions. Longer seasons also allow greater capture of light energy and accumulation of hydrothermal time for both summer and winter crops in double cropping systems, increasing yield potential (Chen et al., 2011; **Figure 4**, point 3). The conservation of soil moisture through late summer in SFZM would also provide a water resource for the establishment of winter crops in double cropping systems, which are currently hampered by growing season duration. By extending the growing season, SFZM has the potential to reduce risks of seasonal crop yield reductions due to delayed harvest under temporal intensification. In addition, the ability of SFZM to enhance soil water conservation could potentially reduce requirements for additional irrigation, as required in some temporally intensified systems (Ray and Foley, 2013).

Temporal intensification may itself also help agriculture become more resilient to climate change. For example, double cropping, facilitated by SFZM, may shift phenologies of some crops, enabling them to avoid peak summer temperatures during critical development phases, when excessive heat can cause severe yield reductions (Seifert and Lobell, 2015). Moreover, SFZM may be particularly suited to support the production-enhancing aspect of temporal intensification because of new technologies for utilizing agricultural biomass from crop residues, and winter-annual cover crops. In the past, biomass crops and crop residues did not contribute to the food supply; however, a variety of new technologies now enable conversion of this biomass into a wide range of foodstuffs for direct and indirect human consumption, as well as biomass feedstocks for bioenergy and bioproducts (Chen and Zhang, 2015). In addition, by enhancing prospects for temporal intensification, SFZM may help reduce the conflict between food and biofuel production by enabling double cropping, potentially supplying both biofuels and food from the same field in the same season (Dale et al., 2010; **Figure 4**, point 3).

Below-Ground Processes in the Virtuous Cycle

By enabling an extension to the period of living plant cover, SFZM can also promote increases in the production of root exudates and crop residues (**Figure 4**, point 4). At the most basic level, the production of microbial biomass is governed largely by input quality and microbial physiological traits, such as microbial C-use efficiency (Sinsabaugh et al., 2013; Wieder

et al., 2014, 2015). Root exudates and plant residues are primary sources of these C inputs, and drive microbial activity, biomass and community composition (Rasse et al., 2005; Hartmann et al., 2009; Rousk and Frey, 2015). Root exudates, in particular, are highly labile, and contain more reduced C compounds and lower C:N ratios, encouraging higher microbial C-use efficiency (Manzoni et al., 2012). Microbial activity is reduced by periods of sustained soil moisture deficiency (Borken and Matzner, 2009), causing reductions in soil nutrient availability (Emmett et al., 2004; Larsen et al., 2011). In addition, repeated wet-dry cycling leads to pulses of soil C and N mineralization, potentially accelerating SOM mineralization over time (Borken and Matzner, 2009). This diminishes soil water holding capacity and increases susceptibility to future soil moisture deficits. Thus, management that produces improved conditions for microbial growth (e.g., adequate water and temperature, plus greater quantities of root exudates), as can be achieved by SFZM, may sustain greater microbial activity and efficiency, thereby enhancing nutrient turnover processes (**Figure 4**, point 5).

Traditional soil models suggest that it is not possible to maintain soil quality under conditions of intensifying production and greater extraction of soil resources, because removal of crop residues and intensification of tillage and fertilization will deplete SOM (Janzen, 2006; Grandy and Robertson, 2007). This may not be the case in agroecosystems managed to create distinct soil functional zones. Existing SFZM systems, such as ridge tillage, have been found to be similar to no-tillage systems in that they support greater microbial biomass than conventionally tilled systems (Angers et al., 1992; Müller et al., 2009b; Zhang et al., 2013). Emerging experimental and theoretical evidence shows that dead microbial biomass (i.e., necromass) is a significant fraction of SOM (Grandy and Neff, 2008; Schmidt et al., 2011; Cotrufo et al., 2013; Frey et al., 2013; Wieder et al., 2014). The continuous and rapid turnover of living microbial biomass can produce, over time, a considerable amount of necromass (Liang and Balser, 2011), which stabilizes SOM (Simpson et al., 2007; Schmidt et al., 2011; Miltner et al., 2012; Cotrufo et al., 2013; Gleixner, 2013; **Figure 4**, point 6).

Although microbial biomass can be rapidly mineralized by soil organisms due to its favorable energy yield and low C:N ratio (Blagodatskaya et al., 2014), microbial necromass and other microbial by-products can also be selectively preserved via interactions with soil minerals and incorporation into soil aggregates (Lützow et al., 2006; Throckmorton et al., 2015; **Figure 4**, point 7). In fact, microbial necromass, metabolites, and decomposition products account for the majority of stabilized SOM (Simpson et al., 2007; Grandy and Neff, 2008; Kleber and Johnson, 2010; Schmidt et al., 2011). The accumulation of stabilized SOM within soil aggregates in turn improves infiltration of precipitation and increases soil water holding capacity (Franzluebbers, 2002; Zibilske and Bradford, 2007; **Figure 4**, point 7). By encouraging the development of greater microbial biomass, SFZM may halt declines of SOM observed under conventional tillage, and instead contribute positively to SOM accumulation and soil structure development while simultaneously supporting greater yield extraction through temporal intensification.

CONCLUDING REMARKS

Development and implementation of novel agroecological management systems that allow increases in provisioning services (yield) while simultaneously enhancing regulating and supporting ecosystem services are urgently needed. As our review shows, SFZM offers a strategy for integrating the production benefits associated with intensively tilled field crop production systems with the soil ecosystem service benefits associated with no-tillage. In short, SFZM offers the potential to achieve the best of both approaches. The soil heterogeneity produced by SFZM enhances soil functional biodiversity, and allows farmers to harness this biodiversity to elicit desirable ecosystem functions at appropriate times and places. This can lead to greater resource-use efficiency and closer synchrony between soil processes and crop physiological demands. Moreover, the ability of SFZM to favorably alter soil hydrothermal properties allows extension of the growing season, both at the beginning and end. This opens opportunities for increasing agricultural production via temporal intensification. Coupled with improvements to soil regulating and supporting services, SFZM therefore offers a vehicle for optimizing multiple ecosystem goods and services in agricultural systems.

Widespread adoption and refinement of SFZM depends on progress on several fronts. Further research on all aspects of SFZM systems will be required to ensure that service delivery can be optimized to meet specific needs of farmers and society in particular cropping systems and geographies. As well, progress on adoption and refinement of SFZM systems is likely to be strongly affected by societal demand for the full range of regulating and supporting ecosystem services that such systems may be able to provide (Mitchell et al., 2016). The case of ridge tillage in maize-soybean production in central North America is instructive: despite its economic viability (Archer et al., 2002), this form of SFZM is not widely used in the US. In this region, it appears that the perceived value of ecosystem services resulting from ridge tillage do not provide a sufficient incentive for its widespread adoption. However, new incentives are appearing, such as the rapidly growing interest in management systems that promote “soil health” (Lehman et al., 2015), increasing innovation in

incentives for agricultural soil C storage (Funk et al., 2015), and more stringent demands for nutrient-use efficiency and other ecosystem services from sustainability-oriented supply chains (Davidson et al., 2014). If there is significant societal demand for the full range of ecosystem services from SFZM, the collective ingenuity of farmers and agricultural engineers can be expected to drive rapid development and implementation of SFZM. This is evidenced by the widespread adoption of zonal tillage techniques in the Central Valley region of California (USA) in response to imperatives to improve resource-use efficiency and environmental performance of production systems in this region (Mitchell et al., 2016).

AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript by reviewing literature, discussing and developing ideas, writing text sections and revising drafts of the manuscript. AW, DK, AD, AG, SH, MH, RK, DM, RS, SS, KS, AY, and NJ developed the initial idea for the manuscript and all contributed to framing and general writing (Abstract, Introduction, SFZM, SFZM and ecosystem services, SFZM and provisioning services, Concluding remarks). PE, LA, and AY contributed to SFZM and Regulating soil services. AJ, ML, and YL contributed to Supporting soil services. AW, AD, AG, RS, and NJ contributed to Virtuous cycles. AW and PE developed **Figure 1**; AG and RS developed **Figure 4**.

ACKNOWLEDGMENTS

This project was supported by an Agriculture and Food Research Initiative (AFRI) Climate Change Mitigation and Adaptation in Agriculture grant (2011-67003-30343), from the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA). The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. USDA is an equal opportunity provider and employer.

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An Unified Framework to Integrate Biotic, Abiotic Processes and Human Activities in Spatially Explicit Models of Agricultural Landscapes

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OPEN ACCESS

Edited by:

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Reviewed by:

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 24 September 2015

Accepted: 18 January 2016

Published: 02 February 2016

Citation:

Vinatier F, Lagacherie P, Voltz M,
Petit S, Lavigne C, Brunet Y and
Lescourret F (2016) An Unified
Framework to Integrate Biotic, Abiotic
Processes and Human Activities in
Spatially Explicit Models of Agricultural
Landscapes. *Front. Environ. Sci.* 4:6.
doi: 10.3389/fenvs.2016.00006

Recent concern over possible ways to sustain ecosystem services has triggered important research worldwide on ecosystem processes at the landscape scale. Understanding this complexity of landscape functioning calls for coupled and spatially-explicit modeling approaches. However, disciplinary boundaries have limited the number of multi-process studies at the landscape scale, and current progress in coupling processes at this scale often reveals strong imbalance between biotic and abiotic processes, depending on the core discipline of the modelers. We propose a spatially-explicit, unified conceptual framework that allows researchers from different fields to develop a shared view of agricultural landscapes. In particular, we distinguish landscape elements that are mobile in space and represent biotic or abiotic objects (for example water, fauna or flora populations), and elements that are immobile and represent fixed landscape elements with a given geometry (for example ditch section or plot). The shared representation of these elements allows setting common objects and spatio-temporal process boundaries that may otherwise differ between disciplines. We present guidelines and an assessment of the applicability of this framework to a virtual landscape system with realistic properties. This framework allows the complex system to be represented with a limited set of concepts but leaves the possibility to include current modeling strategies specific to biotic or abiotic disciplines. Future operational challenges include model design, space and time discretization, and the availability of both landscape modeling platforms and data.

Keywords: conceptual model, human practices, interdisciplinarity, landscape, modeling procedure

INTRODUCTION

The emergence of the ecosystem service concept (Costanza et al., 1997) has ignited interest in the landscape-oriented disciplines and stressed the need for an integrated view of landscapes. As emphasized by Termorshuizen and Opdam (2009), landscapes deliver a wide range of “functions” that can be viewed as “services” when they are valued by humans. According to these authors, the study of landscape services requires an interdisciplinary approach at the landscape scale. Scientists

from various disciplines are encouraged to cooperate in producing a common knowledge base that can be integrated into multifunctional landscape assessments.

Agricultural landscapes are of primary interest since they cover about 38% of the global landmass (FAO, 2011). Although they differ from other landscapes in that they are mainly managed for food or feed production and under strong influence of human activities (e.g., crop allocation, plowing, irrigation, fertilization, ditch digging), agricultural landscapes shelter a large range of processes, which are either abiotic (e.g., runoff, soil erosion, sedimentation and transport by air flows) or biotic (e.g., plant growth, dispersal of organisms, and biodegradation of pesticides). Each process, whether it is biotic, abiotic or human, acts at a given spatial and temporal scale and grain, and the estimation of an ecosystem function is the result of complex interrelations between a set of biotic, abiotic and human processes that need to be analyzed.

Natural processes and human activities in agricultural landscapes have been analyzed within a number of scientific disciplines such as landscape ecology (Burel and Baudry, 2003; Turner, 2005), catchment hydrology (Grayson et al., 2002; Schröder, 2006), landscape-scale pedology (Pennock and Veldkamp, 2006), and landscape agronomy (Verburg and Overmars, 2009; Benoît et al., 2012). These disciplines are built on a common “pattern-process” or “structure-function” paradigm, in which landscape patterns both impact and are impacted by the processes occurring in landscapes. They also share the use of spatially explicit models that account for the spatial variations of biotic or abiotic processes and their interactions with landscape features at different scales (Beven and Kirkby, 1979; Arnold, 1990; Tilman and Kareiva, 1997; Ovaskainen and Hanski, 2004). Indeed, spatially explicit modeling tools are useful for predicting the impact of specific and spatially distributed landscape management actions (Matthews et al., 2007). However, while sharing some common paradigms, the different disciplines have independently developed their own landscape modeling approaches. Each has considered specific landscape features and processes, and in turn has developed specific concepts for representing landscape functioning and to understand ecosystem services provided by landscapes. However, spatio-temporal boundaries of the considered processes or landscape element often differ between disciplines (for example the plot limits are different whether ecological, agronomical or pedological processes are considered). This makes it problematic to couple several abiotic and biotic processes to understand ecosystem services provided by landscapes.

In this perspective paper we suggest an explicit conceptual framework that should help modelers from different disciplines to build spatially and temporally coherent landscape representations that link biotic, abiotic and human processes before implementing their models. The framework is adapted to spatially-explicit models focusing on the impact of spatial arrangement of human practices in landscapes. It provides a generic spatial and functional representation of the main characteristics and related processes of landscapes. We illustrate the potential of this framework on an example of agroecosystem involving several interacting biotic, abiotic and human processes.

Specific questions related to the scientific and technical application of our conceptual framework are addressed in a final section.

PRESENTATION OF THE FRAMEWORK

The framework represents the landscape characteristics and processes with five generic landscape features:

1. Time-variant landscape mosaics, that are composed of Immobile Landscape Elements (ILE) such as fields, ditches, soil units, etc. ILE have a fixed position in the landscape, although their characteristics and spatial extent may change with time. They are defined by their geometry, which forms the geometry of the whole landscape, their position, and a set of properties governing the landscape processes (e.g., hydraulic conductivity and habitat attractiveness). ILE have permanent connections with other ILE, which depend on the spatial arrangements of the mosaics. The ILE properties are variable in time because they are influenced by landscape processes, human activities, and other external conditions.
2. A set of landscape processes that can be either biotic or abiotic and that may have an explicit spatial dimension (e.g., water fluxes in a landscape or insect dispersal) or not (e.g., plant growth). Landscape processes closely interact with the landscape mosaics because they are influenced by the geometry, properties and state variables of ILE (e.g., runoff is influenced by differences in soil infiltration capacity resulting from the various cultivation techniques used across the fields) and may change, in turn, the ILE and their connectivity (e.g., in the case of dispersal for overwintering insect species for which forests offer suitable habitats during winter but not during other seasons).
3. A set of Mobile Landscape Elements (MLE) that represent bodies of matter (e.g., soil material, water bodies volumes, biological individuals, or populations) whose position can vary (they circulate within and between the ILE), and whose characteristics may change with time according to landscape processes, human activities, and other external conditions. Their position and geometry can change with time within the geographical landscape structure provided by the ILE. The characteristics of MLE are described by state variables that can be quite diverse, according to the landscape modeling literature. They can be quantities, concentrations, volumes, or the geographical position of any MLE of interest, like insects or mammals taken as groups or individuals. State variables of MLE differ from previously defined properties of ILE in that (i) their variations in time are faster than those of the properties governing landscape processes (e.g., soil hydraulic conductivity varies more slowly than soil water level), and (ii) they can serve as output or input for further evaluation of landscape services.
4. A set of human activities that may interact with items 1 and 3 above. The impacts of human activities on landscape mosaics are defined by their location (e.g., a farm territory for a farmer), the induced modifications of the geometry or properties of ILE (e.g., building a ditch), and the fluxes

of elements that they affect. Activities are also prone to modify the state variables of the MLE (e.g., irrigation increases soil water content, or pesticides applications decrease insect abundance). Conversely, the geometry and properties of ILE, as well as the status of the MLE, greatly affect human activities (e.g., farmers sow according to soil moisture and apply pesticide in response to pest abundance). Other intrinsic factors affecting human activities, such as personal characteristics of land managers, could be integrated in this component provided that they exhibit spatial or temporal heterogeneities.

5. A set of external conditions that depend on processes taking place outside or at the boundaries of the landscape, and that affect landscape processes by fixing or modifying the landscape boundary conditions. Obvious examples of external conditions are the climatic conditions or biological infestations to which the landscape is subjected.

ILLUSTRATION OF THE FRAMEWORK APPLICABILITY ON AN AGROECOSYSTEM EXAMPLE

In what follows, the conceptual framework is tested against a case study that involves (i) multiple biotic, abiotic and human processes, (ii) variations in space and time of landscape conditions, and (iii) several mutual impacts of actions and feedbacks.

We consider an agricultural landscape located in a Mediterranean area (43° 30'N, 3° 19'E). This landscape includes a variety of land uses, i.e., vineyards, annual crops, fallows and woodlands (Sluiter and Jong, 2006), different landscape infrastructures, i.e., pounds and ditches (Levavasseur et al., 2015), and a complex soil pattern (Coulouma, 2008). We focus our study on three landscape services: water erosion control (especially for the prevention of soil loss by runoff, as in David et al., 2014), water regulation (water provisioning for agriculture, see Levavasseur et al., 2012), and enhancement of remarkable biodiversity (Davies et al., 2008; Herzon and Helenius, 2008) with the provision of corresponding habitats for two endangered animal species (the common toad, *Bufo bufo* and the damselfly, *Calopteryx virgo*).

This case study can be conceptualized according to our framework, as shown in **Figures 1, 2**. **Figure 1** provides an overview of the whole case study. Its conceptualization includes (i) a time-variant landscape mosaics composed of six ILE on which act (ii) three abiotic and biotic landscape processes associated with (iii) five MLE, all being impacted by (iv) four human activities and (v) two external conditions. It must be noted that the first four MLE are those directly related with the targeted ecosystem services, i.e., soil material for water erosion control, surface water for water regulation and toads and damselfly for biodiversity enhancement. We added “plant material” that strongly interacts with the first four MLE. The set of properties and state variables of ILE and MLE for this example can be found in Appendix 1 in Supplementary Material. It indicates that the same property of a given ILE could be

linked to several MLE through their state variables, for example the habitat attractiveness of riparian borders that are linked to toads and damselfly positions, and plant material diversity and abundance.

Figure 2 shows two snapshots of the case study (located in time by arrows in **Figure 1**), i.e., the landscape functioning in mid-winter (**Figure 2A**) and in mid-summer (**Figure 2B**). At each period, a subset of active MLE (pictogram) embedded in ILE (mosaic elements) is considered. ILE can circulate from one element to another, following spatial connections that are defined according to the landscape processes at play (the plain arrows in **Figures 2A,B**). Human activities impacting ILE are represented with dotted arrows. The detailed processes presented in **Figure 2** are described below.

During winter (**Figure 2A**) the climate is characterized by low temperatures and large amounts of rainfall. Low temperatures result in a reduced activity of the fauna and flora (biotic MLE), while the large amounts of rainfall lead to significant surface water flow (abiotic MLE). The water flow is initiated by field overland flow that erodes the topsoil of fields and riparian borders (ILE), thereby impacting the erosion control service. It also impacts the water regulation service by going into ponds or ditches (ILE), and further to a watershed outlet.

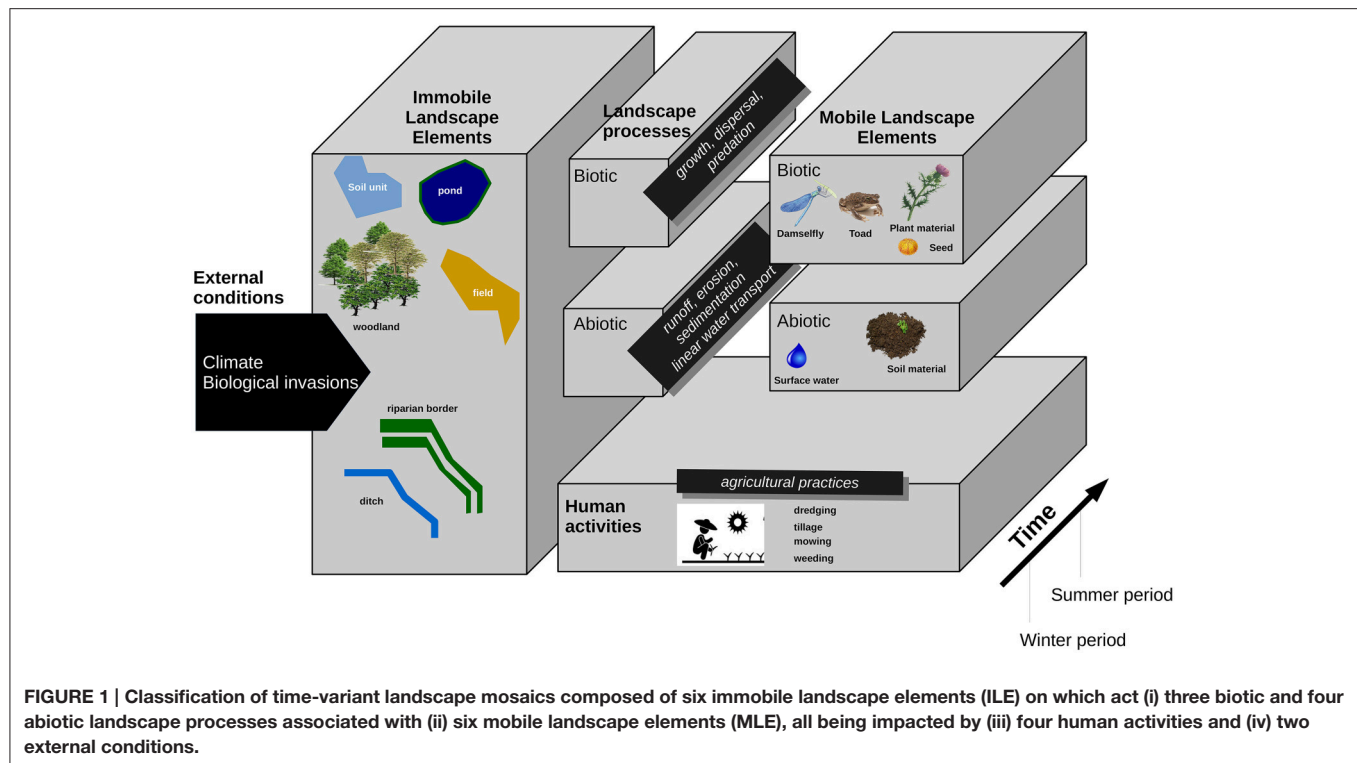
Spatial variations of water flow in the agricultural landscape are driven by a variety of conditions. Field runoff is driven by the soil infiltration capacity, which is itself modulated by the nature of soils and the occurrence of tillage operations. In turn, the water flow in ditches is driven by the slopes and roughness of ditches. The latter is related to the amount and composition of flora in the ditch, which can be modified by dredging, mowing, weeding or burning the ditches.

In summer (**Figure 2B**), the climate is characterized by small amounts of rainfall and high temperatures. Small amounts of rainfall do not induce any water flow (abiotic MLE) while high temperatures result in significant biotic activity (biotic MLE). The common toad and the damselfly move away from their overwintering sites (woodlands) and search for new habitats for mating and egg-laying (riparian borders).

In summer, spatial variations of toads and damselfly populations within the agricultural landscape are driven by several factors. Both organisms may use riparian ditch borders as corridors for dispersal and as shelters. The damselfly density and the number of locations occupied by toads increase faster in areas where plant cover is high, a characteristic linked to the occurrence of ditch dredging, mowing, weeding or burning. The attractiveness of ponds for mating depends on water flow reaching the pond in the previous year.

The functioning of the agricultural landscape is thus characterized by several interactions between biotic and abiotic processes and human activities (**Figure 2C**). Three of them can be cited as examples:

- (i) seed dispersal in winter, due to surface runoff, increases the density of standing plants in the ditches, which will further affect ditch roughness and subsequently limit water flow;
- (ii) water flow in winter induces topsoil erosion in fields that may settle by sedimentation in the neighboring ditches and



thereby decrease their cross-sectional area, which in turn may increase topsoil erosion in the vicinity of ditch banks, due to overflow of the ditches;

- (iii) plant growth in summer influences ditch roughness, which will limit water flow in winter and, beyond a given threshold of rainfall intensity, will induce damaging overflows that will make the farmers more active in weeding, dredging, mowing or burning their ditches, which will in turn limit plant growth.

FURTHER CAPACITIES OF THE FRAMEWORK

Our conceptual framework allows us to represent a complex landscape functioning that includes biotic, abiotic processes and human activity, with a limited number of concepts (ILE and MLE with their associated properties and state variables, respectively). It is the first step of a modeling approach that would take into account the whole complexity described above with a balanced and common representation of biotic, abiotic and human processes that act in this agricultural landscape. The consideration of ILE and MLE in this framework allows a subsequent implementation following an object-oriented programming strategy, i.e., objects defined by classes, and classes inheriting attributes and implementation from pre-existing classes called base classes.

Another added value of this approach is that biotic and abiotic processes are seen through a common framework that is spatially explicit, and sufficiently non-restrictive to withstand retroactions between processes. This approach differs from the current two

ways of coupling multiple processes in landscape models that are found in the literature:

- (i) an unbalanced representation of biotic, abiotic processes and human activities, due to the core discipline of the modelers (Freeman et al., 2001; Gibbins et al., 2001; Nathan et al., 2011; Pazos et al., 2013), which results in representing one-sided relations only and neglecting possible feedbacks;
- (ii) a balanced but oversimplified representation of the systems, using metamodels for example (Harrison et al., 2014). The latter approach was based on a resolution and an extent not able to cope with landscape mosaics of human practices, and therefore cannot simulate emerging properties of the system at landscape level.

A third advantage of our framework is that it matches the current landscape modeling strategies that have been followed by the different disciplines. For example, the framework could challenge our old representations of processes through physical or ecological models. In the example described above, the state variables of biotic populations could be modeled as continuous quantities following mass conservation laws (an Eulerian representation) whereas state variables of abiotic matters could be modeled as discrete elements by adopting an object-oriented view (a Lagrangian representation).

FUTURE CHALLENGES

Developing and assessing the applicability of this framework is the first step toward integrating landscape modeling approaches.

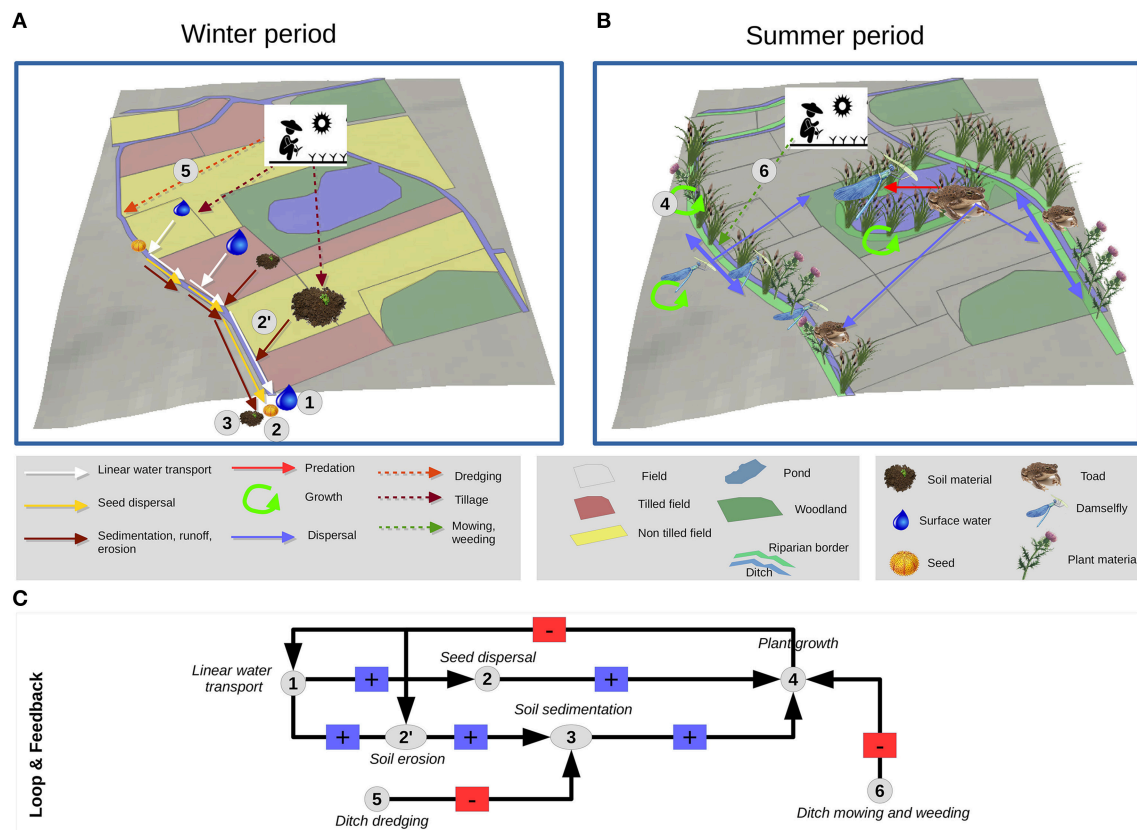


FIGURE 2 | Spatial organization of ILE and MLE during winter (A) and summer (B), with processes indicated by arrows, and positive and negative interactions between processes and between human activities and processes (C). The spatial variability at a given time is represented through variations in state variables attached to MLE (represented by the size of pictogram in A,B), and variations in landscape properties attached to ILE (differences in colors and patterns).

A full implementation of the framework will require five important challenges to be met.

Model Design Challenge

Our conceptual framework should be considered as an initial step toward the integration of landscape modeling approaches. It leaves open a wide range of modeling options that should be addressed by the modeler. The test example presented here illustrates the types of choices that must be made to model an agricultural landscape with a given set of objectives in mind. The identification and selection of landscape processes, ILE and their properties, external conditions, MLE and their state variables, and interrelations will likely differ according to the modelers' objectives and must be clearly defined and justified. Moreover, the numerical complexity and cost of a full coupling of processes, as well as the search for parsimony must be considered. A wide range of studies have simulated complex human-environment interactions that are highly constrained by the spatial and temporal scales chosen in the agent-based platforms, as for example grid base space segmentation and regular time steps (Parker et al., 2003; Matthews, 2006; Caron-Lormier et al., 2009; Schreinemachers and Berger, 2011). There is also a strong

unbalance between the considered processes, depending on the core disciplines of the modelers, such as sociology for agent-based models, ecology (Gibbins et al., 2001; Vinatier et al., 2012) or hydrology (Thomas et al., 2014). Moreover, hierarchical models are rarely considered to simulate landscape processes acting at different spatio-temporal scales.

Computational Challenge

Given the complexity discussed above, it becomes clear that landscape scientists alone cannot translate the present conceptual framework into operational landscape models. We advocate the emergence of landscape modeling platforms (see Sklar, 2007; Verburg and Overmars, 2009; Fabre et al., 2010; Grignard et al., 2013) that will enable an individual scientist involved in the study of a specific landscape process to model its interaction with other landscape processes without being an expert in all landscape disciplines and without being an expert programmer.

Space-Time Challenge

How space and time are discretized will greatly affect the model behavior. Discretization in space should basically account for the geometry of ILE that is considered important for a

given landscape process. However, it may be necessary in some instances to represent the variability of properties within the ILE and to discretize them. This can arise when the ILE heterogeneities in the landscape are very large or when the sole segmentation of ILE leads to too abrupt spatial variations in state variables, generating numerical instabilities in the modeling approaches. Similarly, the properties of time discretization reflect the choices made to account for the dynamics of landscape mosaics and the impact of human activities. Whatever the selected discretization, down or upscaling geostatistical procedures for transferring information between irregularly shaped space-time cells should be developed to ensure the appropriate flow of information within the system.

Data Challenge

The implementation of the proposed conceptual framework in real landscape situations may require a substantial amount of data. Landscape sciences have accumulated a huge repository of knowledge on landscape processes and their interrelations. Despite this, data requirements will remain important, and there is a need for long-term experiments and monitoring at the landscape level, as is currently performed in long-term socio-ecological research platforms, “zone ateliers” and SOERE (Mauz et al., 2012). Beside, landscape modelers require the development of spatial data infrastructures providing basic spatial data for landscape modeling (e.g., digital elevation models, land use maps, etc.) in order to define important landscape elements of the study region and estimate their properties.

Knowledge Challenge

Although this paper insists on the necessity to develop a pluridisciplinary framework for modeling landscape services, it is also important to stress that the definition of landscape processes and MLE, as well as their relation to ILE, suffers from knowledge gaps. This is particularly true for the conceptual representation of biotic processes in landscapes, whose study is more recent

than that of abiotic processes. Contrasting paradigms based on dispersal or niche concepts have been proposed to explain metacommunity assemblages, but there is a need to test these paradigms against empirical data generated from a large number of case studies (Logue et al., 2011).

We are also faced with a lack of generic laws that are needed to model the link between human activities (including agricultural practices) and the properties of the ILE. We need to develop conceptual frameworks that are more accurate than those prevailing in comparative studies of agricultural landscapes, which use broad classes of agricultural systems such as organic and conventional systems (e.g., reviews in Bengtsson et al., 2005; Letourneau and Bothwell, 2008; Garratt et al., 2011).

AUTHOR CONTRIBUTIONS

FV contributed to the redaction of the first draft. PL, MV contributed to the description of the framework. SP, CL, YB, and FL contributed to the redaction of the introduction and conclusion.

ACKNOWLEDGMENTS

The authors thank François Massol, David Bohan, Jean-Christophe Fabre and the Payote network for helpful advice on earlier versions of the manuscript. They also thank the two anonymous referees for helpful comments and suggestions on the manuscript. INRA and its Ecoserv metaprogram are gratefully acknowledged for funding the work presented in this paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fenvs.2016.00006>

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Conflict of Interest Statement: 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.

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Making the Most of Our Land: Managing Soil Functions from Local to Continental Scale

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OPEN ACCESS

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Aristotle University of Thessaloniki,
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 31 August 2015

Accepted: 04 December 2015

Published: 22 December 2015

Citation:

Schulte RPO, Bampa F, Bardy M, Coyle C, Creamer RE, Fealy R, Gardi C, Ghaley BB, Jordan P, Laudon H, O'Donoghue C, Ó'hUallacháin D, O'Sullivan L, Rutgers M, Six J, Toth GL and Vrebos D (2015) Making the Most of Our Land: Managing Soil Functions from Local to Continental Scale. *Front. Environ. Sci.* 3:81. doi: 10.3389/fenvs.2015.00081

The challenges of achieving both food security and environmental sustainability have resulted in a confluence of demands on land within the European Union (EU): we expect our land to provide food, fiber and fuel, to purify water, to sequester carbon, and provide a home to biodiversity as well as external nutrients in the form of waste from humans and intensive livestock enterprises. All soils can perform all of these five functions, but some soils are better at supplying selective functions. Functional Land Management is a framework for policy-making aimed at meeting these demands by incentivizing land use and soil management practices that selectively augment specific soil functions, where required. Here, we explore how the demands for contrasting soil functions, as framed by EU policies, may apply to very different spatial scales, from local to continental scales. At the same time, using Ireland as a national case study, we show that the supply of each soil function is largely determined by local soil and land use conditions, with large variations at both local and regional scales. These discrepancies between the scales at which the demands and supply of soil functions are manifested, have implications for soil and land management: while some soil functions must be managed at local (e.g., farm or field) scale, others may be offset between regions with a view to solely meeting national or continental demands. In order to facilitate the optimization of the delivery of soil functions at national level, to meet the demands that are framed at continental scale, we identify and categorize 14 policy and market instruments that are available in the EU. The results from this inventory imply that there may be no need for the introduction of new specific instruments to aid the governance of Functional Land Management. We conclude that there may be more merit in adapting existing governance instruments by facilitating differentiation between soils and landscapes.

Keywords: Functional Land Management, ecosystem services, policy, soil functions, sustainable intensification

INTRODUCTION

Context: Demands on Land

In 2014, the United Nations (UN) revised their projections for population growth: the world's population is no longer expected to stabilize after 2050, but is now forecast to continue to grow and approach 11 billion people by 2100 (Gerland et al., 2014). These figures will certainly fuel the debate on whether the world is “running out of land” (Keesstra et al., 2015). Indeed, the UN Food and Agriculture Organisation (FAO) estimate that the world may need to increase food production by 60% over the period 2005–2050, in order to feed a predicted population of more than nine billion (Alexandratos and Bruinsma, 2012).

However, not only do we expect the world's agricultural land to provide a nutritious diet for all, we also expect it to secure clean water, to sequester and store carbon, to host biodiversity and provide a home for our organic waste (Montanarella and Vargas, 2012; Schulte et al., 2014). At the same time, the required increase in agricultural productivity is projected to add further stress to the availability and usage of these ecosystem services. For example, agricultural greenhouse gas (GHG) emissions currently account for 10–12% of global GHG emissions (Smith et al., 2007). The Organisation for Economic Cooperation and Development (OECD) projects that agricultural emissions of methane and nitrous oxide will increase in absolute terms, in tandem with increased agricultural production, whilst carbon dioxide emissions arising from land use changes are projected to fall (Marchal et al., 2012).

In addition, agriculture is the world's largest user of freshwater, accounting for 70% of global freshwater use (Evans, 2009), although there are large differences in water use between regions and farming systems. A large proportion of the projected increase in global agricultural production is likely to be derived through new irrigation initiatives; the FAO estimates that irrigation will increase by 11%, predominantly in areas with precipitation deficits (Bruinsma, 2009). This increase will most likely have negative consequences for local groundwater or riverine water balances where over-abstraction occurs. This may be further exacerbated by climate change: for example, Zhao et al. (2015) modeled irrigation requirements across Europe for six major crops under climate change scenarios, and predicted increased requirements for drier regions. In moister regions of Europe, the interface between agriculture and water is predominantly defined by the impact of farming on the quality of drinking water and the ecological quality of connected water bodies (Withers and Haygarth, 2007). Here, the prevention of eutrophication, caused by excess nutrient losses from land to water, represents one of the main challenges to sustainability (e.g., Schulte et al., 2006).

The relationship between agriculture and biodiversity is complex: occupying 38% of the world's land surface (FAOSTAT, 2013), agriculture simultaneously provides habitats for biodiversity and competes for space with non-agricultural ecological habitats (FAO, 2015). If increased food production were to be achieved through an expansion of land area used for agriculture, this would pose challenges to the preservation of ecological habitats and their associated biodiversity. However, a sole reliance on “intensification” could equally pose challenges

to species and habitats associated with land currently used for extensive agricultural production (e.g., Robinson and Sutherland, 2002; Tscharnkte et al., 2005).

The magnitude and complexities of these interrelated challenges are such that no less than six of the 17 Sustainable Development Goals of the United Nations (<http://sustainabledevelopment.un.org/>) are devoted, either directly or indirectly, to the sustainable management of land, namely:

- Goal 2: End hunger, achieve food security and improved nutrition, and promote sustainable agriculture;
- Goal 6: Ensure availability and sustainable management of water and sanitation for all;
- Goal 7: Ensure access to affordable, reliable, sustainable, and modern energy for all;
- Goal 12: Ensure sustainable consumption and production patterns;
- Goal 13: Take urgent action to combat climate change and its impacts;
- Goal 15: Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.

These multiple and interrelated challenges have given rise to a situation where land is becoming an increasingly constrained natural resource, particularly in the context of land degradation through erosion (Ye and Van Ranst, 2009; Sauer et al., 2011) and soil sealing, with the latter being responsible for a “loss” of about 20 million ha of agricultural soils each year (Nachtergaele et al., 2011). This necessitates prudent management from the local to continental and indeed global scale. The urgency of this need is exemplified by recent concerns about “land grabbing,” i.e., the process where land in a country is “secured” by investors to produce primary goods commonly destined for other jurisdictions (see Cotula et al., 2009, for a balanced review). These concerns on the finite nature of land as a resource have now brought the significance of soil science sharply back into focus.

Managing Soil Functions

In 2006, the European Commission published the EU Thematic Strategy for Soil Protection (European Commission, 2006a), which outlined the suite of functions that soils perform for humankind, as well as the threats to this functionality. The subsequent proposal for a Soil Framework Directive (European Commission, 2006b) built on the concept of threats to soil quality, which included erosion, contamination, loss of organic matter, loss of biodiversity, compaction, salinization, flooding, landslides, and sealing. The Directive proposed a suite of actions to mitigate against these threats, aimed at maintaining soil quality throughout the EU. However, this exclusive focus on threats, while only hinting at soil quality as a prerequisite of the utility function of soils, led to resistance from stakeholders, including the farming community (COPA-COGECA, 2008). This, as well as a multitude of unrelated political considerations, ultimately

resulted in the withdrawal of the proposed Directive in 2014 as part of the EU REFIT initiative (European Commission, 2014).

However, these developments have not reduced the urgent need to explore how we can safeguard our land resource for the provision of food and other ecosystem services (Maes et al., 2012). Indeed, in 2014 the Commission stated that it remains committed to the objective of the protection of soil and will examine options on how to best achieve this. This commitment is reflected in the *2011 Resource Efficiency Road Map—Europe 2020 Strategy*. As part of this strategy, EU policies must account for their direct and indirect impact on land use in Europe and globally by 2020. Responding to the Road Map, the European Commission is working on a Communication on “*Land as a resource*,” that will provide a new framework for sustainable and appropriate land management across the EU. This Communication is aimed at (a) accelerating actions in the context of valuing land as a resource for ecosystem services, (b) filling the gap between demand and availability of land, and (c) setting synergies and trade-offs between competing land uses and functions (Deloitte, 2014).

To inform this process, Schulte et al. (2014) proposed “Functional Land Management” as a more utilitarian framework, aimed at optimizing the delivery of five main soil functions (Figure 1):

- (1) Primary productivity: the provision of food, feed, fiber and fuel;
- (2) Water purification and regulation: the ability of soils to purify and regulate water for human consumption and maintenance of ecosystem integrity;

- (3) Carbon storage and regulation: the ability of soils to store carbon for (a) partial offsetting of GHG emissions and (b) regulation of biological and physical soil processes;
- (4) Provision of a habitat for biodiversity, both below-ground and above-ground diversity;
- (5) Cycling and provision of nutrients, specifically the ability of soils to provide a sustainable home for external nutrients such as those derived from landless farming systems (e.g., pig and poultry farms), as well as sewage sludge and other organic waste products.

Intrinsically, all soils can perform all of these functions, but some soils are better at some functions than others (e.g., Ghaley et al., 2015). The relative suite of functions depends primarily on land use and management, and an indicative illustration of this dependency was provided in the original paper (Schulte et al., 2014). In addition, the relative supply of each soil function depends on soil properties. For example, in Atlantic climates, soil drainage is the predominant soil property that defines the functionality of soils (Schulte et al., 2012). Using this pedo-climatic zone (based on the delineation by Metzger et al., 2005) as a case study, the interdependencies between soil functions, land use and drainage were explored in detail by Coyle et al. (2015), who developed conceptual models for each of the soil functions, culminating in a matrix that illustrates the relative ability of contrasting soils and land use combinations to supply each of the five soil functions (Figure 2).

Functional Land Management aims to optimize, rather than maximize, the supply of each of the soil functions in order to meet the societal demands for all five functions simultaneously. In the original paper, Schulte et al. (2014) illustrated this by assessing the supply and demand for the five soil functions at a national scale, using Ireland as a case-study. However, they noted that this national assessment “masked” significant variation, both in the supply and demand for individual soil functions at local scales, and was therefore not yet fit-for-purpose to inform policy making. Therefore, there is a need to assess the spatial scale at which both the demand for, and the supply of each soil function applies.

Objective

Therefore, with a view to informing the forthcoming EU Communication on *Land as a Resource*, the objective of this paper is two-fold:

- (1) First, we explore the spatial variability and patterns of the supply of each of the five soil functions, using Ireland as a national example. We subsequently assess the spatial scale at which each of the demands for these soil functions apply, from the local scale to national or continental scale.
- (2) Secondly, we examine the options for governance of Functional Land Management, specifically how the supply of soil functions can be managed to meet the multitude of demands, as framed by EU policies. We make an inventory of policy and market instruments that are available for governance, and bring these together into a framework for policy-making that guides and connects decision making across spatial scales.



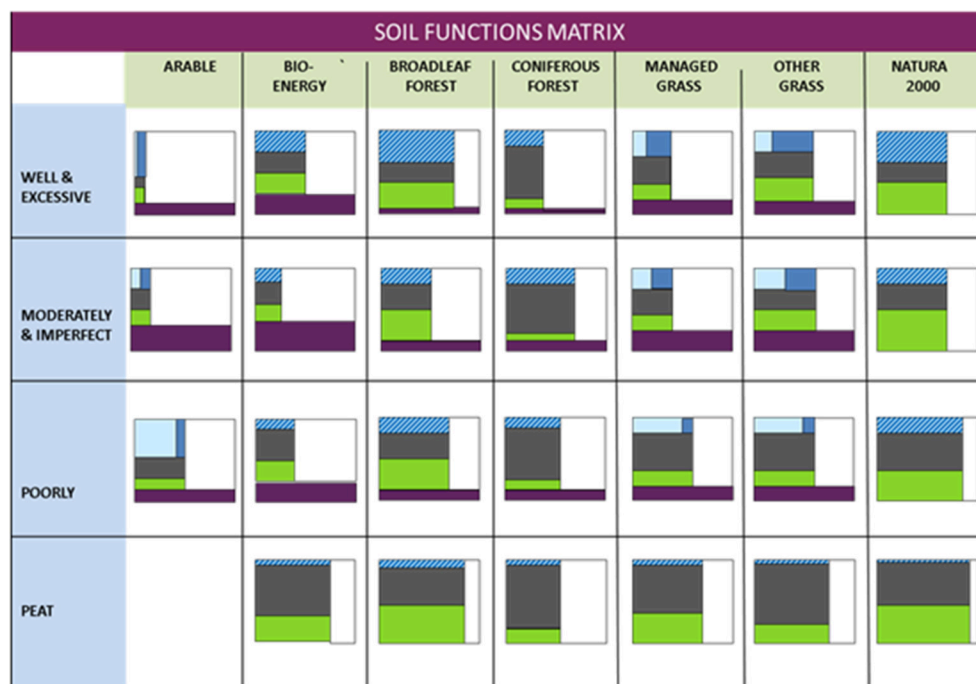


FIGURE 2 | Soil Functions Matrix (adapted from Coyle et al., 2015) illustrating the supply of the five soil functions (the size of the five boxes) in relation to land use (horizontal axis) and soil drainage (vertical axis). White boxes indicate primary production; blue, water purification and regulation; black, carbon storage and regulation; green, provision of a habitat for biodiversity; purple, cycling of nutrients.

MATERIALS AND METHODS

Supply of Soil Functions

To quantify the spatial variation in the supply of each of the five soil functions in Ireland, we used the partial proxy-indicators previously published by Schulte et al. (2014) and Coyle et al. (2015) for Atlantic climates. These were: (i) carrying capacity for primary productivity; (ii) denitrification capacity for water purification; (iii) potential carbon sequestration; (iv) habitat type in relation to species richness, abundance and biomass, and (v) the capacity to sustainably process slurry from pig farms and/or sewage sludge for the function nutrient cycling.

To facilitate mapping of the supply of soil functions, we intersected the new 1:250,000 Indicative Soil Drainage Map of Ireland (Schulte et al., 2015) with the 1:250,000 Land Use Map of Ireland (O'Sullivan et al., 2015), in order to derive a map of combinations of land use and drainage, equivalent to those used in **Figure 2** above. The Soil Drainage Map was based on the new 3rd generation 1:250,000 Soil Map of Ireland (Creamer et al., 2014). For each of the five functions, we calculated z-scores (Wagg et al., 2014) to derive a normalized weighting for each combination of land use and drainage category, based on the relative proportionalities of **Figure 2**.

Demands for Soil Functions

In order to assess spatial variation in the demand for each of the soil functions, we first conducted a policy review to frame these demands, as defined by EU policies. In the absence of a Soil

Framework Directive, there is no single overarching EU policy that comprehensively defines the demand for each of the soil functions. Instead, this demand is framed by a large number of EU policies. **Figure 3** lists the most pertinent of these policies, the associated soil functions of relevance, and the spatial scale to which each policy applies. We then defined (partial) proxy-indicators for each of these demands. The selection of these proxy-indicators was guided by the availability of spatial data.

The main EU policy driver for increased primary productivity in an Irish context is the abolition of the milk quota in 2015, as part of the new Common Agricultural Policy (CAP). This is of particular relevance to Irish farming, where many dairy farms have hitherto been constrained by quota rather than by land. Food Harvest 2020 (Department of Agriculture, Food and the Marine, 2010) is the industry strategy, supported by the Irish Government, to grow the agricultural sector, with a specific emphasis on increasing the volume of national milk output by 50% in the period 2015–2020. This ambitious volume target for the dairy sector is currently framing the demand for increased primary productivity. As most of the new volume is derived from existing dairy farms, rather than from new entrants (pers. comm. S. Molloy, Director of Strategy, Glanbia), we chose the density of dairy cows per District Electoral Division as the proxy-indicator for the spatial patterns of the demand for increased primary productivity.

The Nitrates Directive (EU, 1991) is currently the main policy that frames the demand for denitrification, as it requires groundwater nitrate concentrations to remain below 50 mg l⁻¹.

EU Policy Driver	Function(s) of relevance ¹	Scale at which demand is framed
Common Agricultural Policy (CAP)	P	EU
Areas of Natural Constraint (ANC)	P	EU / National
Greening Measures	C H	Ubiquitous
Nitrates Directive	W N	Ubiquitous / Regional
Water Framework Directive	W H	River Basin District
Habitat & Birds Directive	H	Multiple scales
Agri-Environmental Schemes	W C H	Farm / regional
EU 2030 Climate and Energy Framework	C	EU + National targets
Sewage Sludge Directive	N	Regional

1. P = primary productivity, W = water purification, C = carbon sequestration, H = habitat provision, N = nutrient cycling

FIGURE 3 | Inventory of EU policy drivers that frame the demand for the five soil functions (illustrated by the colored boxes), and the spatial scale to which each of these policies applies.

This is enforced throughout the EU by imposing a maximum annual application rate of organic nitrogen at 170 kg N ha^{-1} (equivalent to the annual excretion from c. two livestock units (LSU) per hectare) in nitrate vulnerable zones (NVZs). Ireland is one of the MS that has adopted a “whole-territory approach” to the concept of NVZs, which means that the restrictions apply to all farms throughout the country. Ireland currently has a derogation that allows for stocking rates up to 250 kg N ha^{-1} , where farmers implement a field-level nutrient management plan based on soil analysis (Government of Ireland, 2009). In this context, we chose stocking rate as the proxy-indicator that frames the policy demand for denitrification.

The demand for carbon sequestration and regulation is framed by two policies: (1) the “Greening Requirements” under Pillar 1 of the CAP and (2) the EU Climate and Energy Framework 2030. The Greening Requirements require, *inter alia*, that soil organic carbon contents are maintained in excess of 2% (Department of Agriculture, Food and the Marine, 2009; Spink et al., 2010) with a dual purpose of maintaining soil quality and preventing GHG emissions in the form of carbon dioxide. However, this latter objective is likely to be addressed more comprehensively in the EU Climate and Energy Framework for 2030. This framework is on track to depart from its predecessor (the Climate and Energy Package 2020), in that it is likely to allow for the Land Use, Land Use Change and Forestry (LULUCF) Sector to be accounted for, subject to constraints, in efforts to reduce national GHG emissions (European Council, 2014). This means that carbon sequestration may provide a pivotal pathway to meeting national ambitions on emissions trajectories. When adopted, the framework will set emission reduction targets for individual MS, disaggregated by the Emissions Trading Sector (ETS) and the non-Emissions Trading Sector (non-ETS). No additional spatial disaggregation will be applied by EU policies. Given that the carbon content of most soils in Ireland is well in excess of 2%, we chose the Climate and Energy Framework 2030, with its national targets for emissions reductions, as the main driver of the demand for carbon sequestration.

The demand for biodiversity is framed by multiple policies that include the Habitats Directive (EU, 1992), Birds Directive (EU, 2009), Water Framework Directive (EU, 2000), the

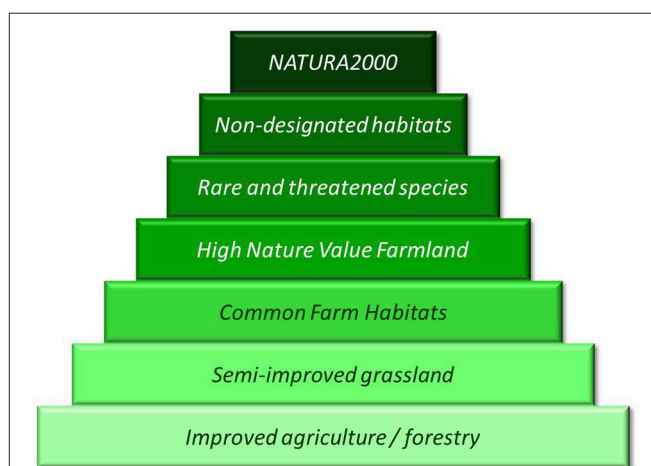


FIGURE 4 | Hierarchy of farmland ecosystems, ranked by the stringency of demands for their maintenance and protection

(O’Uallachain, 2014). Ecosystems at the bottom of the pyramid are most common and geographically widespread, but demands for their protection are least stringent, whereas ecosystems at the top occur in specific, smaller areas, but are subject to the most stringent demands.

EU Biodiversity Strategy (EU, 2011), Greening Requirements (CAP Pillar 1) and agri-environment schemes (CAP Pillar 2). Previously, in a presentation to national policy makers of the Joint Oireachtas Committee on Agriculture, Food and the Marine, O’Uallachain (2014) ordered these into a “hierarchy of demands,” ranging from the most stringent demands that apply to Natura 2000 sites to the least stringent demands that apply to improved grasslands outside protected areas (Figure 4). We adopted this hierarchy to frame the policy demand for biodiversity, using data from O’Sullivan et al. (2015), the National Parks and Wildlife Service (NPWS), the Land Parcel Identification System (LPIS) from the Department of Agriculture, Food and the Marine, and CORINE land cover data provided by the Environmental Protection Agency.

The demand for nutrient cycling is framed by two EU policies, namely the Nitrates Directive (EU, 1991) and the Sewage Sludge Directive (EU, 1986). These two Directives frame the demand

for sustainable disposal and application of intensive animal enterprise (i.e., pig slurry and poultry manure) and sewage sludge, respectively. This has proven particularly challenging for pig slurry, which has a relatively low dry matter content and high water content, resulting in the need to transport large volumes of dilute material. Therefore, the sustainable disposal and application of pig slurry on land that has the capacity (biophysically and legally) to process the additional external nutrients, is primarily constrained by the costs of transport. In this context, we selected the area required for the sustainable disposal of slurry from the pig farms in the country as the relevant proxy-indicator for nutrient cycling, constraining the application rate to the maximum rate of $19 \text{ kg phosphorus ha}^{-1} \text{ year}^{-1}$. In addition, we added the area required to sustainably dispose of the volume of sewage sludge produced by towns and cities with a population in excess of 5000 inhabitants.

Governance Tools for Functional Land Management

To meet the second objective of the paper, we conducted a literature review to derive an inventory of governance instruments available to policy makers to manage the supply of soil functions at local and national scales to the demands for these functions from local to continental scales. These instruments were categorized along two axes, i.e., (1) the spatial scale at

which each of the instruments applies and (2) the nature of implementation (market-driven, mandatory, voluntary).

RESULTS

Supply of Soil Functions

The five supply maps show a high degree of granularity in the supply of each of the soil functions (Figure 5). Whilst some regional patterns emerge, particularly for the provision of primary production, biodiversity, and nutrient cycling, the supply of soil functions is primarily defined by local soil and land use characteristics (inset in the carbon sequestration supply map).

Demand for Soil Functions

Contrastingly, there are marked differences in the spatial patterns of the demands for the five soil functions, as illustrated by the bottom row of maps in Figure 5, with spatial variation manifesting itself from small scale (primary productivity) to regional scale (nutrient cycling) and national scale (carbon sequestration).

In most regions, the supply of primary productivity exceeds demand, which suggests that sufficient capacity is available for the increased productivity projected in the Food Harvest 2020 Strategy. This supports the earlier conclusions by Schulte et al. (2014) who reported average national stocking rates of 1.2 LSU

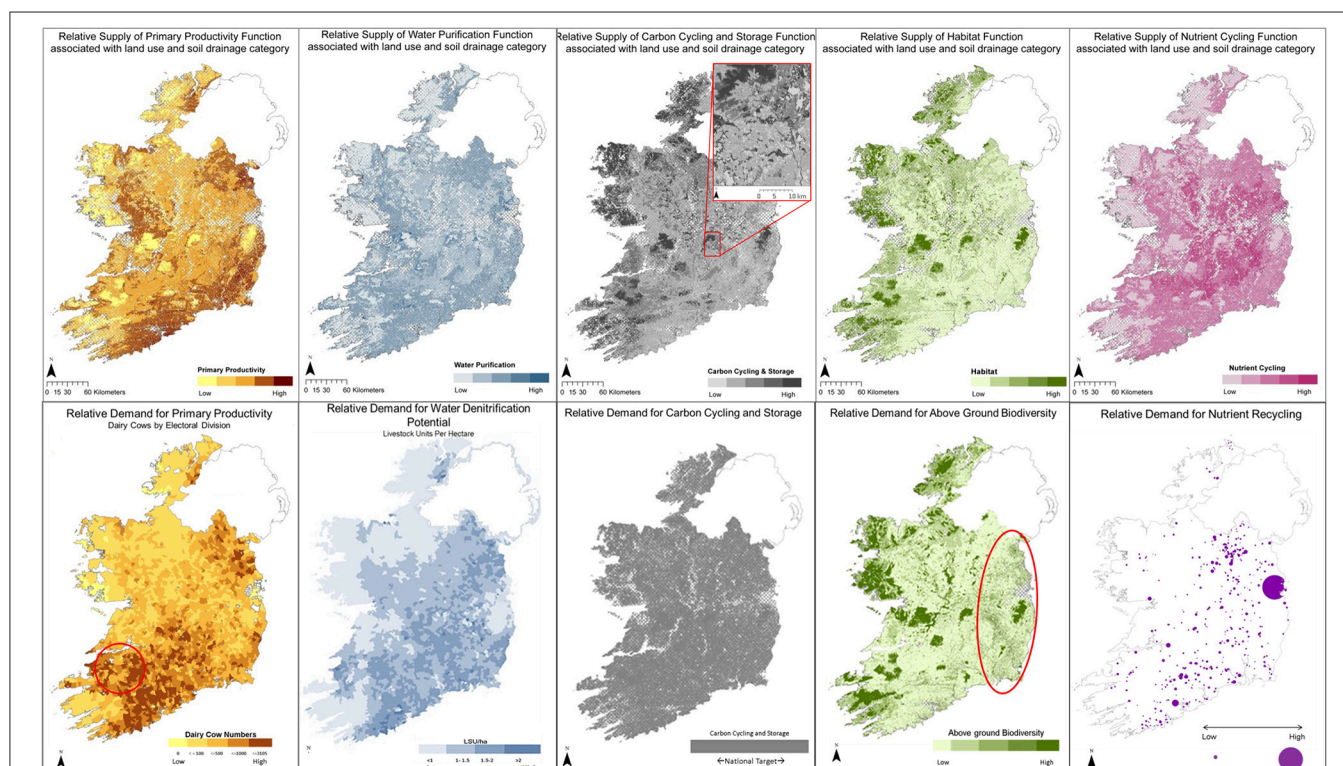


FIGURE 5 | Indicative maps of the normalized supply (top row) and demand (bottom row) for the five soil functions, from left to right: primary productivity, water purification, carbon sequestration, biodiversity, nutrient cycling. The red circle and oval indicate areas, discussed in the text, where the demand for primary productivity and biodiversity, respectively, may exceed the current supply of these two functions.

per hectare, well below the carrying capacity of 1.5–1.8 LSU per hectare of most soils. A comparison of the supply and demand maps for primary productivity shows that the demand for increased productivity is highest in areas with a generous supply of this function (e.g., in the South-West, East, and North-East of Ireland), i.e., on soils that are traditionally classified as “good agricultural soils” (Lee and Diamond, 1972). However, there are exceptions, most notably in some South-Western regions (red circle), which are characterized by poorly-drained soils. In this region, a higher demand for primary productivity may only be met by an increased supply through the installation of artificial drainage systems, which moves the soil to a different drainage category; this will be considered in further detail in the Discussion.

The supply of denitrification, as a partial proxy for the function “water purification” is high in all regions, and adequately meets the demand that groundwater nitrate concentrations remain below 50 mg l⁻¹. This is indeed reflected in the very high proportion of land (99%) in Ireland that is in compliance with this demand (Byrne and Fanning, 2015).

Whilst the potential supply of the function “carbon sequestration” differs significantly between soil types, land use types, and management, the demand for this function applies to national scale only, as it is at this scale where GHG reduction targets will apply. **Figure 5** illustrates this by assigning a uniform color to map the demand for carbon sequestration (note that waterbodies and urban areas show up in different shading). The EU 2030 Climate and Energy Framework has thus far only specified targets for GHG reductions at EU scale. Over time, this is expected to translate into national targets, but there are many difficulties associated with further downscaling these targets to regional or farm scale, including high transaction costs and concerns regarding the equitability of “carbon quota” in the context of diverse pedo-climatic environments (Teagasc, 2011).

A comparison of the supply and demand for the function “habitat for biodiversity” shows that one of the most stringent demands, i.e., the designation of Natura 2000 sites, has been met, although significant challenges remain in relation to the requirement to ensure favorable conservation status for these habitats (Schulte et al., 2014). Additional, more recent demands arising from the “Greening measures” of the CAP, include the requirement for Ecological Focus Areas (EFAs) to be implemented on arable land (red oval), which has traditionally been associated with a low supply of habitat.

The maps of the demand vs. supply of the soil function “nutrient cycling” show that the demand is low in comparison to the potential supply. This is a reflection of both the low pig population and human population in Ireland, compared to some of the other EU MS. Furthermore, the demand is strongly regional. The size of the circles represents the total area required to dispose of the organic nutrients. These circles represent an “ideal” scenario, where all of the land within the circle is suitable, and landowners are willing, to import the manure. In reality, finding suitable spreadlands can involve long travel distances.

DISCUSSION

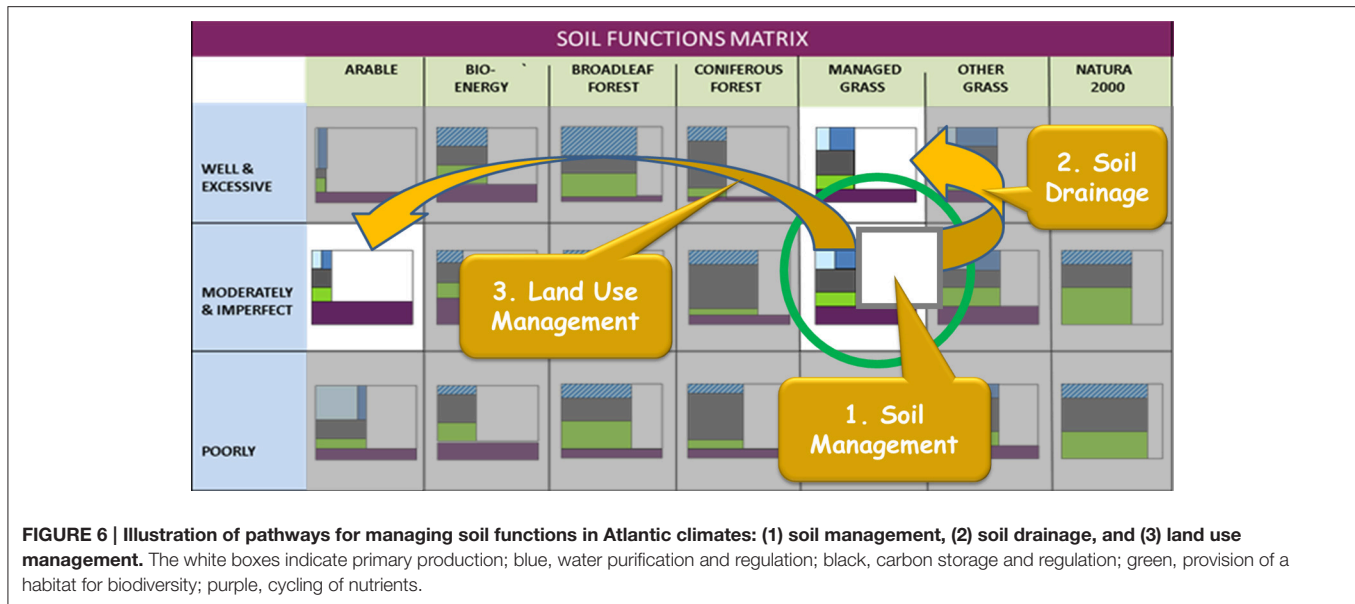
Maximizing or Optimizing Soil Functions?

The concept of Functional Land Management neither seeks to maximize a unique soil function, nor all soil functions of all soils at the same time. Indeed, the latter has proven to be impossible, given that not only synergies, but also trade-offs exist between some of the soil functions (Power, 2010). For example, efforts to increase primary productivity on wet soils through the installation of subsurface drainage systems may reduce the capacity of a soil to perform the function of carbon sequestration (O’Sullivan et al., 2015). Instead, Functional Land Management constitutes a systems approach aimed at managing soils (and in a wider context: managing land) in such a way that the demands for soil functions are balanced and met everywhere, thus building on the recommendations by Bouma et al. (2012) and Kibblewhite et al. (2012). In light of the spatial variation of both the supply and demand for soil functions, this involves a process of “optimizing,” rather than “maximizing” soil functions, subject to the balances of local demand and supply.

This subtle change in focus may help us to further develop the concept and definition of “Soil Quality.” Almost two decades ago, the Soil Science Society of America (1997) defined Soil Quality as “The capacity of a specific kind of soil to function within natural or managed ecosystem boundaries, to sustain biological productivity, maintain environmental quality, and promote plant and animal health.” Whilst this definition provides a utilitarian and hence pragmatic approach to soil quality that centers on “functionality,” it is unbounded in that it does not provide a benchmark or scale for the assessment of soil quality. In light of the aforementioned potential for trade-offs and synergies between soil functions, there is merit in adding the clause “The capacity of a specific kind of soil to *provide functions to meet demands*, within...,” which would add a benchmark for assessment. Additionally, it would introduce a degree of elasticity, since Soil Quality would no longer depend solely on soil properties and processes, but could also vary through a change in demand(s). Whilst this elasticity may be challenging from a scientific perspective, it provides a useful feedback mechanism to policy formation, specifically in the formulation of demands on soil functions. Put simply: it could help in assessing whether proposed demands are achievable or not.

Pathways for Matching Supply to Demand

When considering practical approaches to matching supply with demand, we can distinguish three contrasting pathways. In **Figure 6** we illustrate these pathways with a case-study of a grass-based dairy farm located on a moderately-drained soil (green circle), where the farmer is planning to increasing primary productivity in response to the abolition of the EU milk quota. The first pathway to meet this “demand” for increased production involves optimizing selected soil functions at a local scale through soil management practices that change the *dynamic* soil properties, such as soil nutrient concentrations. Ideally, such good practices may augment a specific soil function without impacting on the others (the “larger white box” in **Figure 6**). For example, nutrient management planning based



on soil testing may increase the potential productivity of soils, without compromising other functions such as water purification (Murphy et al., 2015). Data from Ireland shows that as few as 10% of agricultural soils have optimum pH levels, as well as optimum phosphorus and potassium concentrations, which demonstrates the potential offered by such simple and affordable measures (Wall et al., 2015). Other examples of this pathway include an increase in the use of animal manures in arable farming (at the expense of fertilizer usage), to augment the functions carbon sequestration and nutrient cycling (e.g., Lal, 2004). When managed correctly, without associated yield reductions or pressures on aquatic ecosystems, this would result in selective increases of the “black box” and “purple box,” respectively, in Figure 6.

The second pathway involves interventions aimed at manipulating *static* soil properties in order to enhance one or more soil functions. In our case study of Atlantic climates (Figures 2, 6), this could involve the installation of subsurface drainage systems to change the drainage capacity and moisture dynamics of a soil, indicated by the second arrow in Figure 6; in other pedo-climatic zones, other types of interventions may be more appropriate. This pathway generally involves a local trade-off between soils functions. For example, in Atlantic climates, the installation of drainage systems typically reduces the prevalence of soil saturation and hence results in longer growing and grazing seasons (Tuohy et al., 2015). However, in some cases this increased productivity may come at the expense of the function water purification (e.g., Jahangir et al., 2012), as drainage systems increase the hydrological connectivity between pressure and aquatic receptors (Haygarth et al., 1998; Uusitalo et al., 2000). In addition, drainage of wet soils (which typically have high carbon contents) may lead to large carbon dioxide emissions, induced by oxygenation (Kechavarzi et al., 2010). O’Sullivan et al. (2015) showed that the cost:benefit ratio of this trade-off between the soil functions primary productivity

and carbon sequestration differs significantly between soil types and meso-climatic conditions, and concluded that the aggregate merits of drainage interventions should be considered site-specific.

The third pathway refers to land use change (Arrow 3 in Figure 6), in this example a change from grassland to arable silage (e.g., forage maize). Similar to the second pathway, land use change typically results in a trade-off between functions. For example, the plowing and conversion of moderately-drained grassland to arable land may increase primary productivity, but this is likely to be at the expense of the capacity of the soil to perform the functions of water purification (Schulte et al., 2006) and regulation (Palmer and Smith, 2013), carbon sequestration (Lal, 2004) and habitat provision for biodiversity (Brussaard et al., 2007; Van Eekeren et al., 2008). It is important to consider that such trade-offs are not necessarily undesirable, provided that they do not irreversibly impede the potential of soil to perform other functions. For example, intensification of primary production may be desirable where the soils have “spare capacity” for water purification, and could be preferable to an alternative scenario of “expansion” of the agricultural production platform into surrounding areas with respect to the functions biodiversity and carbon sequestration, if these latter areas are of high nature value. Contrastingly, intensification through land use change may be undesirable on soils where the supply of the purification capacity cannot meet water quality requirements.

The Role of Scale

The spatial scale to which these demands apply will determine the extent to which the supply of soil functions can be offset between soils or regions. Soil differs from commodities such as air and water, in that most soils are owned and as a result managed at local scale. At the same time, we have seen that the demands for soil functions may range from local to continental scales, presenting landowners with a myriad of considerations.

For example, the Nitrates Directive requires that groundwater nitrate concentrations are maintained below 50 mg l^{-1} . This demand is ubiquitous and implies that the soil function of water purification cannot be “traded” between fields, farms or regions, where one location would compensate for the failure of another to provide clean water. Contrastingly, the Water Framework Directive has a regional focus, which allows for a degree of offsetting between land areas at a catchment scale. This means, on the one hand, that the impact of individual farms on the ecological status of surface waters may be “diluted” over a catchment. Conversely, this impact may be compounded by non-agricultural sources of nutrients, e.g., waste water treatment plants (Vrebois et al., 2015). Due to the directionality of river systems, the demand for the function “water purification” may be spatially separated from the demand. This may constrain options in relation to offsetting, especially in complex river systems.

At the other extreme, the demand for soils to sequester carbon applies ultimately to a global scale, as the atmospheric impacts are independent of the location of the sequestration. In practice, the demand for management of GHG applies at national scale. Either way, this larger scale allows for a degree of offsetting between soils or regions, where one soil may compensate for low rates of sequestration in other locations. This means that it may not be efficacious to translate national carbon sequestration targets into a requirement for every farmer to offset his/her emissions through land management. Instead, it may be more prudent to focus the delivery of this soil function on soils less suitable for the primary productivity function, e.g., by incentivizing farm forestry on these latter soils.

The same principles of offsetting apply, to some extent, to primary productivity, facilitated by the emergence of a global food system, although cognizance must be paid to the multiple advantages of regional supply management (e.g., Sonnino, 2013), which have been further highlighted by geopolitical developments in recent times.

The demands for the remaining two soil functions, biodiversity and nutrient cycling, apply at intermediate scales. The demand and supply dynamics of nutrient cycling are primarily governed by transport considerations (Fealy and Schröder, 2008), which restricts the options to match demand and supply at the regional scale. Demands to designate and protect biodiversity cut across multiple scales, as these pertain to the protection of individual rare species (local scale), entire habitats (regional scale), and the protection of species throughout their natural range (national scale) (Noss, 1990).

Incentivization and Implementation

The need for coherent management of soil functions across scales, from local to national and continental scale, does not equate to a requirement for top-down “zoning” of land for individual functions: the granularity of the supply of soil functions emerging from **Figure 5** demonstrates that this may be unachievable in any case. Instead, individual soil functions may be incentivized by a range of instruments that have a long history within the framework of the EU CAP. These include mandatory incentives such as the cross-compliance and greening requirements of CAP Pillar 1, as well as voluntary

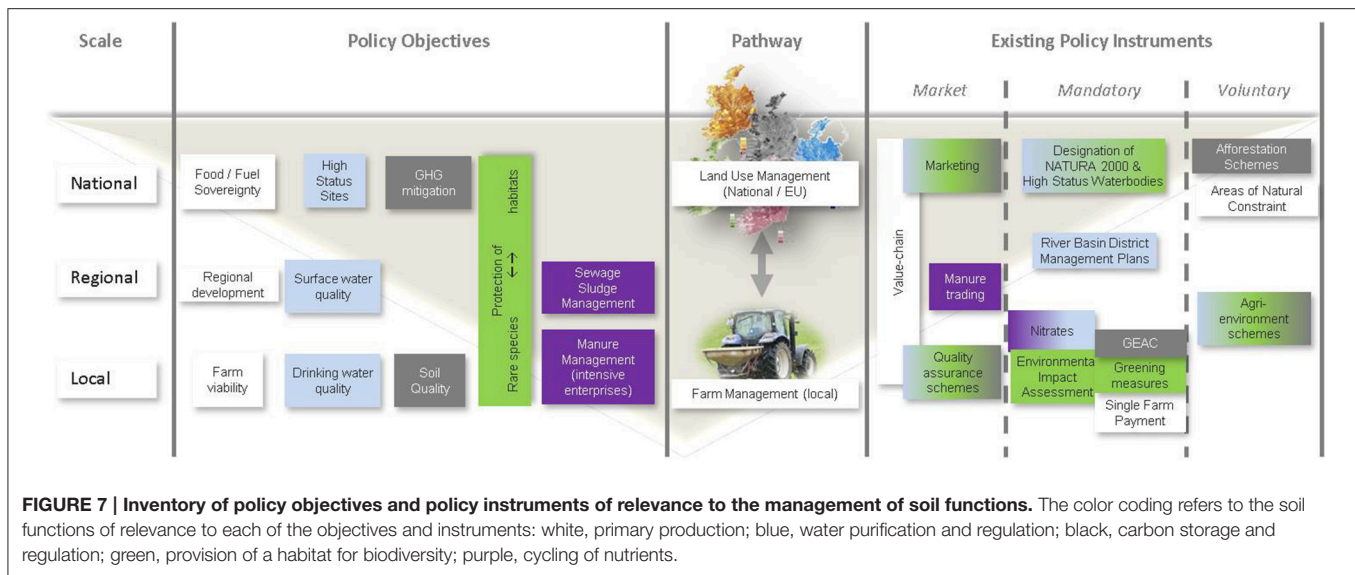
incentives such as agri-environment schemes and the new Areas of Natural Constraints (ANC) of CAP Pillar 2. In **Figure 7**, we have categorized these instruments into market driven incentives, mandatory policies, and voluntary schemes, alongside the policy objectives, and have identified their corresponding spatial scale of application.

Policies at national scale focus on the management of land use. Examples include the mandatory requirement for MS to designate NATURA 2000 sites (Habitats and Birds Directives) and High Status Waterbodies (Water Framework Directive). Joint implementation of these two requirements allows for synergy and a seamless approach. For primary productivity, the main instrument to regulate demand is the market, specifically following the decoupling of payments from production levels, and the recent abolition of EU milk quota. At the same time, the Pillar 2 payments for ANC are an example of a voluntary scheme to compensate farmers for reduced capacity of soils to deliver primary productivity.

We could identify few mandatory policy instruments that apply consistently at regional scale across the EU, with the notable exception of the River Basin District Management Plans, which are the main instrument for managing water catchment areas as part of the Water Framework Directive. However, at national scale, other environmental policies are also governed and implemented at a regional to local scale, specifically in decentralized MS. For example, in federate Belgium, agricultural policies and the implementation of the WFD, Urban Waste Water Directive and Habitat Directive are all governed at regional scale (Flanders, Walloon, Brussels). Similarly, voluntary policy instruments may be implemented at regional scale. One example are the agri-environment schemes of the Po Valley, which is characterized by the most intensive and productive agricultural systems of Italy, which typically deliver a low “supply” of the function “Habitat for biodiversity.” Apart from river networks and Natura 2000 sites, permanent grasslands represent the most relevant additional hotspots of biodiversity (Assolari et al., 2004), and higher soil carbon stocks (Gardi et al., 2002). The historic extent of this land use type has been significantly reduced over the last 50 years. Several regions have now tailored the agri-environment schemes financed by CAP, in order to provide economic incentives for the conservation of permanent grasslands.

Finally, at the local scale, a continuum of incentives are focussed on the augmentation of selective soil functions, ranging from the mandatory requirement to maintain soil carbon contents in excess of 2% in order to maintain soil quality, greening requirements, nitrates regulations and mandatory Environmental Impact Assessment, to voluntary agri-environment schemes and quality assurance schemes aimed at enhancing local soil management practices to ensure delivery of the water purification and habitat functions (e.g., <https://www.glanbiaingredientsireland.com/sustainability/farm>).

A comparison of the policy objectives (left-hand side of **Figure 7**) and policy instruments (right-hand side of **Figure 7**) shows that, in principle, policy makers have a wide menu of instruments at their disposal to incentivize soil and land management to meet policy objectives. In other words: the



individual policy tools for Functional Land Management are available for the management of soil functions at the appropriate scale. Some of the policy tools are “joined up” in addressing multiple soil functions: for example, through the mechanism of cross-compliance, the Good Agri-Environmental Condition (GAEC) requirements and the Greening requirements are tied into eligibility for the full Single Farm Payment (SFP) scheme. However, there are an equal number of relevant instruments that operate in isolation of each other. For example, soils that are less suitable to provide food are currently being identified as part of the re-delineation of ANC. This offers opportunities to target afforestation incentives to such areas, to negate competition for land between the production of food and fiber. Another example is the Environmental Impact Assessment, a mandatory requirement for large-scale land-management interventions, such as the installation of drainage systems. This assessment only applies to the soil function of habitat provision. In light of the aforementioned potential impact of soil drainage on the carbon sequestration function, there may be room for a more holistic approach that addresses multiple soil functions.

Secondly, most of these policy instruments were developed in response to a multitude of diverging policies that originate from more than one body of the European Union or its MS. As a result, these instruments are often developed and administered by multiple actors that include multiple ministries and local authorities, and in the case of quality assurance schemes, also commercial entities such as primary processors and retail chains. Anecdotal evidence suggests that this has imposed complex and at times confusing requirements for record keeping on landowners. Whilst this may be considered necessary from a regulatory perspective, this complexity carries the risk that landowners will view “sustainable land management” from a perspective of compliance, rather than an opportunity “to make the most of our land,” even where negative financial trade-offs are compensated for by support mechanisms. In any case, financial benefits, either short-term or long-term, may not always be

self-evident to farmers. For example, the benefits of enhanced carbon sequestration as a climate change mitigation measure, accrue at societal level, rather than at farm scale (Gutzler et al., 2015).

Finally, many if not most of the instruments do not account for differences between soils in their capacity to supply soil functions. This implies that these instruments are implicitly based on the “old” concept that seeks to maximize either one, or all soil functions simultaneously. If we accept that the availability of land, be it at local, national or continental scale, is a limiting factor in meeting the demands for all major soil functions simultaneously, then we need policy instruments that aim to optimize, rather than maximize, the supply of soil functions, which means that differences between soil types need to be accounted for. To date, this has been impeded mainly by the unavailability or incompleteness of soils data at national scales in many EU MS, compounded by inconsistencies between datasets. However, many of these challenges are currently being addressed in countries with poor soil information, as part of the ongoing new delineation of ANCs across the EU (Eliasson et al., 2010), which requires the consistent mapping of soil properties within and between MS (Van Orshoven et al., 2012). Upon completion, this could also be used to customize and fine-tune the other policy instruments to take account of differences between soils.

Application at European Scale

In this paper, we used Ireland as a case-study to illustrate the variety of scales to which the demand for soil functions may apply, and how an integrated approach to policy formation, across spatial scales, is required to optimize the supply of soil functions to meet societal demands for food, clean water, climate change mitigation, biodiversity and the sustainable management of manure and sewage. At this point, it is prudent for us to emphasize that we developed this case-study merely for the purpose of illustrating our framework. Whilst the apportioning of the supply of soil functions in **Figure 2** is based on an extensive

literature review and conceptual modeling (Coyle et al., 2015), this is not yet underpinned by direct empirical data. Instead, this is the subject of the current SQUARE (Soil QUALity: Assessment and REsearch) project, in which we are measuring the supply of the five soil functions at more than 40 sites across Ireland, with a view to validating the matrix presented in **Figure 2** (see <http://www.teagasc.ie/soil/square/>).

Similarly, the main purpose of the maps in **Figure 5** is to illustrate the high level of small-scale granularity in the supply of soil functions, and the diverging spatial scales at which each of the demands for soil functions applies. While these maps may be used to aid the identification and interpretation of large-scale geographical patterns, they are not appropriate for aiding management decisions at local (i.e., farm) scale. These maps were derived from the 1:250,000 third generation soil map of Ireland. At that scale, polygons represent associations of soil types that occur together in a landscape but may exhibit diverging properties. Therefore, local management of soils requires knowledge of local soil types, which can only be derived through direct observations.

In addition, while we are confident that our five soil functions comprehensively cover the main demands on land in Europe, our choice of proxy-indicators provide only a partial representation of these soil functions. For example, in this paper we chose the denitrification capacity of soil as a partial proxy-indicator for the function water purification and regulation, for illustrative purposes only. A full analysis would have to include additional proxy-indicators, for example to quantify the capacity of soils to adsorb phosphorus or agro-chemicals, or the capacity of soils to regulate water quantities and prevent droughts and/or floods. Indeed, other MS may need different or additional proxy-indicators in order to adequately frame both the supply and demand for soil functions. In our example, we used soil drainage as the dominant soil property that, together with land use, determines the supply of soil functions in Atlantic climates. In other biogeographical zones in Europe, the supply may depend primarily on other soil properties (e.g., pH, texture) or other land use types that we did not include in our case-study (e.g., viticulture). The identification of common criteria (soil properties) for the delineation of ANC (Van Orshoven et al., 2012) may prove a useful step in identifying the most appropriate soil properties for each biogeographical zone.

The demand for soil functions may equally differ between localities, regions and countries. For example, there are large differences in nitrogen surplus across Europe (Leip et al., 2011a) and regions with larger nutrient surpluses will see a higher demand on soils for water purification (e.g., Uwizeye et al., accepted). Areas with large urban conglomerations or intensive farm enterprises (e.g., pig or poultry industry) may put a larger demand on soils for nutrient cycling, for example in Denmark (Dalgaard et al., 2011) and in the Flanders region of Belgium (Van der Straeten et al., 2010). Contrastingly, countries with proportionally high agricultural gaseous emissions may emphasize the demand on soils to sequester carbon (e.g., Leip et al., 2011b; Department of Agriculture, Food and the Marine, 2015; Embassy of France, 2015).

This upscaling of the concept of Functional Land Management to EU scale is the primary objective of the

new Horizon 2020 project LANDMARK (LAND Management: Assessment, Research, Knowledge base), a consortium of 22 partner institutes from 14 EU countries plus Switzerland, China and Brazil. For full details follow @LANDMARK2020 on Twitter.

CONCLUSIONS

Soils provide multiple ecosystem services for society, which can be grouped into five “soil functions”: primary productivity, water purification and regulation, carbon sequestration, providing a home for biodiversity, and recycling of nutrient. While all soils can perform all functions at the same time, the relative suite of functions that can be supplied by a soil depends primarily on land use and soil properties. Given the fine-grained spatial variability of both land use and soil properties, the supply of the five soil functions may vary from field to field.

Most of the societal demands for soil functions emanate from European policies relating to agriculture and the rural environment. These demands may apply to very different spatial scales: for some functions, such as the provision of clean groundwater, the demand applies at the field scale, while for other functions, such as mitigation of GHG emissions through carbon-sequestration, the demand applies at national or even continental scale. This wide range of scales of application has profound implications for the management for soil functions, specifically with respect to the “tradability” of the supply of soil functions between regions: some functions must be managed (e.g., by individual farmers) at a local scale, whereas other may be “traded” between regions.

The concept of Functional Land Management seeks to optimize, rather than maximize, the supply of soil functions to meet societal demands, without resorting to “top-down zoning” of land management. Across the EU, we identified 14 types of governance instruments for land management at local, regional and national scales, that could in principle be used to facilitate Functional Land Management. Most of these address the management of soil functions indirectly, and do not account for differences between soils in their capacity to supply soil functions, due to a historic knowledge deficit of European soil resources at a relevant scale. The current process of re-delineation of ANC may provide a harmonized platform to rectify this and customize policy instruments for these differences. Such customization could negate the need to develop new government instruments, and could instead aid the alignment of existing instruments with a view to developing a coherent approach to land management.

ACKNOWLEDGMENTS

This study was conducted as part of the LANDMARK (LAND Management: Assessment, Research, Knowledge Base) project. LANDMARK has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 635201. The SQUARE (Soil QUALity Assessment and REsearch) project, mentioned in the text, is funded by the Research Stimulus Fund of the Department of Agriculture, Food and the Marine of the Government of Ireland.

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Collaboration between Mountain and Lowland Farms Decreases Environmental Impacts of Dairy Production: The Case of Swiss Contract Rearing

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OPEN ACCESS

Edited by:

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 16 July 2016

Accepted: 31 October 2016

Published: 16 November 2016

Citation:

Marton SMRR, Lüscher G,
Corson MS, Kreuzer M and Gaillard G
(2016) Collaboration between
Mountain and Lowland Farms
Decreases Environmental Impacts of
Dairy Production: The Case of Swiss
Contract Rearing.
Front. Environ. Sci. 4:74.
doi: 10.3389/fenvs.2016.00074

Mountain farming areas are associated with high nature value and offer attractive landscapes, but farming in these areas is less viable than farming in more favorable regions. Consequently, there is a threat of land abandonment. Additionally, due to lower productivity of mountain farms, their products often bear a higher environmental burden than those from other areas. An optimal division of labor between mountain farms and farms in more favorable regions based on comparative advantages could help maintain attractive landscapes and reduce environmental impacts of agricultural production. An established Swiss contract rearing system, in which dairy farms from the agriculturally favorable lowlands collaborate with heifer rearing farms in the mountains, represents a promising approach for such a division of labor. In this system, the intensive phase of dairy production is performed in the lowlands, while the less intensive phase is performed in the mountains. Here, we analyzed a sample of 16 farms to compare the contract rearing system to a situation in which both, mountain and lowland farms produce milk and rear their own restocking animals. We performed a life cycle assessment to quantify environmental impacts of the dairy production systems, assessing environmental impacts both per kg of milk and per hectare of agricultural area. This assessment was supplemented with analysis of the workload of the farms, since increased work efficiency is one reason that farmers engage in contract rearing. Workload was calculated with a workload budgeting tool. We found that collaboration reduced environmental impacts as well as the workload per kg of milk. Collaboration had no effect on environmental impacts per hectare of agricultural area or the workload on lowland farms, while on mountain farms the environmental impacts and workload were reduced. In particular, reduction in environmental impacts of mountain farms is expected to foster the high nature value of this farmland and the

provisioning of important ecosystem services. This case study of a contract rearing system thus illustrates how collaboration based on comparative advantages can benefit both environmental impacts of agricultural products and the high nature value of agriculturally less favorable farmland.

Keywords: contract rearing, less favored areas, natural constraints, dairy, life cycle assessment, biodiversity, workload

INTRODUCTION

Farming activities in mountainous regions face natural constraints that inhibit high productivity. Instead, such areas are often of high nature value and feature attractive landscapes. They provide important ecosystem services, such as maintenance of genetic resources, storage, and purification of water, as well as cultural and heritage services (Grêt-Regamey et al., 2012; Plieninger and Bieling, 2013). This high nature value farmland has been shaped through traditional low-intensity agricultural systems (Lomba et al., 2014). Today, such areas are threatened by two trends: intensification and land abandonment. Land abandonment mainly results from lower economic viability (Strohbach et al., 2015). To address the reduced economic viability and prevent land abandonment, the European Union's Common Agricultural Policy (CAP) and agricultural policies in European countries outside the EU, such as Switzerland or Norway, have defined plans to support farming activities in these areas (MacDonald et al., 2000; Marriott et al., 2004; Gellrich and Zimmermann, 2007). Since environmental conditions do not allow intensive agricultural production and specific policy measures may limit high-input farming, agricultural land management in areas with natural constraints is often more environmental friendly, with lower fertilizer or pesticide use per hectare (Rudow, 2014). On the other hand, because of the lower productivity of the land, from a life cycle assessment (LCA) perspective, the provision of agricultural goods from these regions is less eco-efficient than in the lowlands. Per unit of product, foods produced in the mountains usually cause higher negative environmental impacts, such as higher global warming potential per kg milk or meat (Hörtenhuber et al., 2010; Ripoll-Bosch et al., 2013). This results in a trade-off between maintaining agricultural production to preserve scenic landscapes with high value semi-natural habitats, and providing agricultural products with a lower environmental impact.

To address this trade-off, the most suitable production systems for such areas have to be identified and, in parallel, environmental impacts of products from these systems must be optimized. An approach focusing only on comparing absolute results of production systems from favorable and less favorable regions could be too narrow, since the chances of identifying a product that is produced more efficiently in areas with natural constraints are rather low. A more promising

approach is inspired by the classic economic theory of trade and comparative advantage (Deardorff, 2014). It focuses on possibilities for division of labor between regions with different natural conditions. By considering environmental impacts of a set of products, it is possible to identify those for which the disadvantage of the region with natural constraints is less pronounced than that for other products from this set, i.e., in which mountain farms have a comparative advantage. In consequence, more favorable regions will have a comparative advantage for production activities for which the disadvantages of the region with natural constraints are more pronounced. An example of division of labor between two regions with different climatic and topographic conditions can be found in Switzerland. Swiss lowland farms generally have agriculturally favorable conditions and invest in grassland-based animal production and crop production. In contrast, mountain farms are mainly restricted to grassland based systems due to steep slopes and a shorter vegetation period. Although dairy farming is practiced in both regions, mountain farms do not compete well with lowland farms. Compared to lowland farms, mountain farms have lower income (Roesch, 2012), and milk with higher environmental impacts per kg (Alig et al., 2011). One reason for the lower performance of mountain farms is the lower nutritive quality of home-grown feed, which, when given alone, is not sufficient for today's high-genetic-merit dairy cows (Horn et al., 2013). In contrast, lowland farmers often perceive their forage quality as being too high for their young stock (M. Tanner, 20 October 2015, pers. comm.).

As early as in the 1960s, farmers from the cantons of Thurgau and Grisons, Switzerland, developed a contract rearing plan that took advantage of the different production conditions on mountain and lowland farms. In this plan, dairy farmers from the lowlands sell their female dairy calves to mountain farmers, who then rear them and sell them back to the lowland farmers shortly before calving. Accordingly, the animals spend the less intensive phase of their life on mountain farms, and the more intensive phase, i.e., the productive phase, on lowland farms, which can offer feed of higher quality. The system remains popular, mainly in these two cantons, but it has spread to other regions as well. It is based on a standardized contract, and prices are renegotiated once a year by a delegation of lowland and mountain farmers. This guarantees a fair pricing system and a legal framework that makes the system easily applicable for farmers. In a previous assessment, this collaborative production system was analyzed based on simulated farms that represented typical Swiss lowland and mountain dairy farms (Marton et al., 2016b). It was shown that collaborative production had environmental advantages compared to a system in which dairy farms rear their own

Abbreviations: CFC-11, Trichlorofluoromethane; FPCM, Fat and protein corrected milk; IDE, International Dairy Federation; KCl, potassium chloride; LCA, Life cycle assessment; LCI, Life cycle inventory; LCIA, Life cycle impact assessment; SALCA, Swiss agricultural life cycle assessment; TAN, Total ammonia nitrogen; UAA, Usable agricultural area.

young stock. However, this kind of assessment with simulated farms is based on many assumptions that might not reflect the real situation. For example, it was assumed that yields and production efficiency per hectare and per cow were the same on collaborating and non-collaborating farms within each region. Thus, the analysis based on simulated typical farms considered only the benefits due to the comparative advantage of each region. However, collaboration may also affect farm efficiency, since collaboration allows farms to specialize in individual aspects of the dairy production system, e.g., milk production itself or rearing of young stock, which was not considered in simulated farms. For the present study, we tested whether the benefits of the contract rearing system observed for the simulated farms are also valid for real farms, and whether the specialization due to collaboration creates further efficiency gains and reduces the environmental impacts of milk production even more. Besides its effect on environmental impacts of milk, specialization was also expected to affect environmental impacts directly on farms. For instance, by outsourcing the less-intensive young stock and keeping only the more-intensive dairy cows, lowland farms might increase adverse environmental impacts per hectare of usable agricultural area (UAA). On mountain farms, specialization in heifer rearing could have the opposite effect, i.e., reduce environmental impacts per hectare of UAA. Furthermore, it is expected that division of labor has an impact on farmers' workload, since labor constraints are considered an incentive for contract rearing (Olynk and Wolf, 2010). In the present study, we therefore compared environmental impacts and workload of collaborative and non-collaborative dairy production using data from real commercial farms. Specifically, we tested the following hypotheses:

- (1) Collaboration leads (a) to intensification and thus to higher environmental impacts per hectare of UAA on lowland farms and (b) to extensification and thus to lower environmental impacts per hectare of UAA on mountain farms. Intensification in this context is defined as an increase in inputs per ha, while extensification corresponds to a reduction in inputs per ha.
- (2) Environmental impacts per kg of fat- and protein-corrected milk (FPCM) produced in the overall system is reduced through collaboration.
- (3) Workload is lower on collaborating farms than on non-collaborating farms.

MATERIALS AND METHODS

Farming Systems and Study Region

We compared two farming systems: collaborative (contract rearing) and non-collaborative. Both systems consisted of dairy farms in the lowlands and the mountains. In the collaborative system, lowland farms concentrated on milk production; female dairy calves designated for restocking were sold to mountain farms when weaned. Mountain farms reared the animals and sold them back to lowland farms when the heifers were close to calving. In the non-collaborative system, dairy cows spent their entire lives on the same farm, i.e., both lowland and mountain farms kept productive dairy cows and young stock for restocking.

Following the previous assessment based on simulated typical Swiss dairy farms from the lowland and mountain regions (Marton et al., 2016b), the present study focused on assessing single real farms to verify or disprove the indications obtained from the farm simulations. The farms analyzed were located in the cantons of Thurgau and Grisons, the two cantons that first adopted the contract rearing plan (**Figure 1**). Both cantons still have many farms that do not participate in contract rearing, which allowed comparison of the collaborative and non-collaborative systems under similar climatic and topographic conditions. Thurgau is a relatively small canton, with ~50% of its area as UAA, mostly in the lowlands, which corresponds to 4.8% of the UAA of Switzerland. Thurgau contains 6.6% of Swiss dairy cows, which produce 7.7% of the milk sold in Switzerland. Grisons is the largest Swiss canton. Due to its location in the center of the Alps, only 8% of its area is used for agriculture, corresponding to 5.2% of the UAA of Switzerland. In addition, 23% of the area of Grisons is considered alpine agricultural area. By law, this area may only be used as pasture during the summer (alpine summer-pasture). Grisons contains 2.9% of Swiss dairy cows, which produce 2.1% of the milk sold in Switzerland (TSM, 2013; Swiss Federal Statistical Office, 2016).

Farm Data and Characteristics

Sixteen dairy farms from the two cantons were assessed; Thurgau represented the lowland and Grisons the mountain region. In each region, four farms were collaborating in contract rearing (hereafter "collaborating farms"), and four farms were not participating in contract rearing (hereafter "non-collaborating farms"). Collaborating and non-collaborating dairy farms were randomly selected from those that offer apprenticeship positions. Since authorization to do so requires additional training for the farmer, we assumed that the farms in the sample were more advanced farms, i.e., farms that apply good management practices and are well-informed about new technical developments.

Data on farm characteristics and agricultural practices were collected during farm visits or provided directly by the farmers. Data collection was based on two datasets developed within the EU FP7 project CANTOGETHER. One set contained data needed for LCA (Teuscher et al., 2014), and the other contained supplementary agronomic and economic data (Regan et al., 2016). **Table 1** gives an overview over some farm characteristics. Farms in the sample were larger and had more animals than the average dairy farm in Switzerland, which had (in 2012) 24 ha UAA and 23 dairy cows. Except for one mountain farm, milk yield per cow also lay above the Swiss average of 6000 kg of milk (TSM, 2013). On lowland farms, most forage was home-grown, mainly grass, and whole-crop maize (silage or dried pellets). Most concentrate was purchased; only a small percentage was home-grown. In addition to producing feed, five lowland farms also grew cash crops. Mountain farms were grassland-based, produced mostly home-grown forage, and purchased all concentrate.

All mountain farms, and one collaborating, and one non-collaborating lowland farm, sent animals to alpine summer-pasture. To represent this phase (~100 days), we collected data

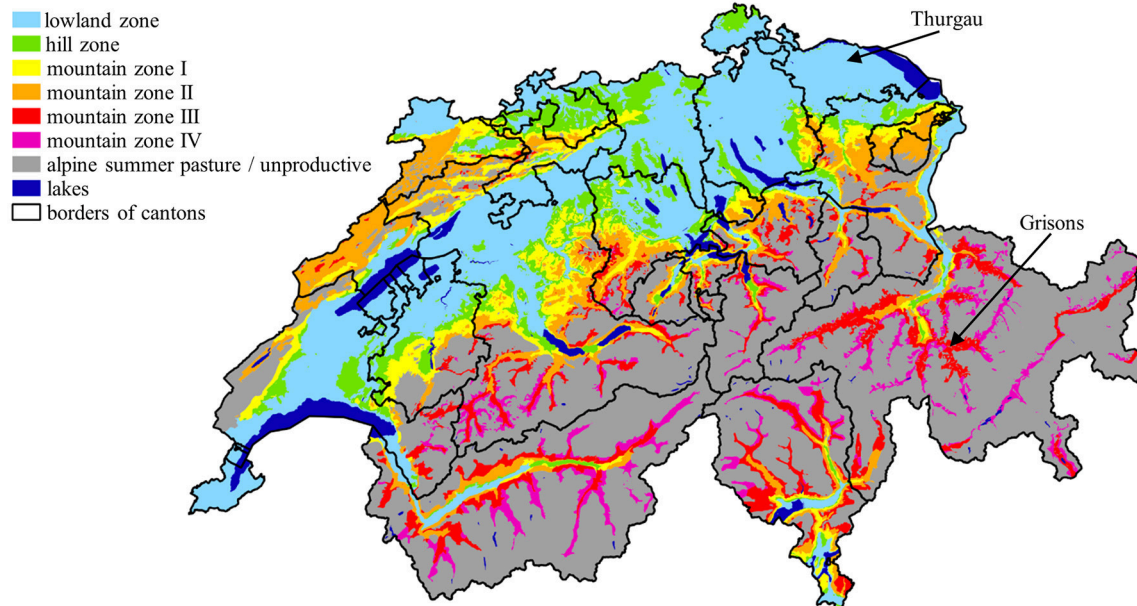


FIGURE 1 | Map of agricultural production zones in Switzerland and locations of the cantons Thurgau and Grisons, where the studied lowland and mountain farms were situated (Federal Office for Agriculture (FOAG), 2015; swisstopo, 2016).

from two alpine summer-pasture farms in Grisons and averaged these data for further calculations.

Most farms collaborated with more than one farm from the other region: mountain farms collaborated with 3–15 lowland farms, which collaborated with 1–5 mountain farms. Since all farms were selected randomly, there was no effective link between collaborating lowland and mountain farms. To simulate collaborative dairy production among the farms in the sample, we combined each collaborating lowland farm with each collaborating mountain farm, resulting in a set of 16 combinations. Farms were combined based on the lowland farm's need for heifers and the mountain farm's production of heifers. For example, if the lowland farm needed four heifers per year and the mountain farm produced 12 heifers per year, then one-third of the mountain farm's heifer rearing enterprise was added to the lowland farm to include the outsourced restocking phase in collaborative dairy production. To compare this collaborative system to non-collaborative dairy production, we combined the non-collaborating dairy farms from the two regions so that the ratio of lowland to mountain UAA corresponded to the median land-use ratio of the two regions in the collaborative system (3.5:1). See Supplementary Tables 1, 2 for details about these combinations. Each non-collaborating lowland farm was combined with each non-collaborating mountain farm, resulting in a set of 16 combinations, which created an equal basis for comparison.

Environmental Assessment

We used LCA to compare environmental impacts of (1) farms within the two regions and (2) milk produced in collaborative and non-collaborative dairy production systems. Life cycle

assessment not only considers environmental impacts occurring directly on a farm, it compiles all environmental impacts of products along the whole value chain. This is typically performed in four steps: goal and scope definition, life cycle inventory (LCI), life cycle impact assessment (LCIA), and interpretation of LCIA results (ISO, 2006; Hellweg and Milà i Canals, 2014).

Goal and Scope Definition

To compare environmental impacts within mountain and lowland regions, the functional unit was one hectare of UAA used for the dairy enterprise during 1 year. This functional unit relates to the farm's function as a provider of ecosystem services, such as maintenance of water quality (van der Werf et al., 2009). The dairy enterprise comprised all farm activities linked to dairy production, e.g., management of dairy cows and restocking animals, production of feed for these animals, and use of buildings and machinery. System boundaries were defined as “cradle to farm gate,” including all environmental impacts caused by the dairy enterprise itself and by all upstream processes linked to production and supply of inputs (e.g., fertilizers, purchased feed), infrastructure and machinery (Figure 2). Seven farms in the sample grew cash crops, which connected the cash-crop and dairy enterprises, since the by-product straw from the former was used as bedding in the latter, and some manure produced in the latter was used as fertilizer in the former. For straw, we performed economic allocation between straw and cash crops to allocate part of the cash-crop area to the dairy enterprise. To account for manure spread on areas allocated to the cash-crop enterprise, we used system expansion, since this method has previously been compared to other approaches and was identified as the

TABLE 1 | Farm characteristics; median and mean deviation of the median per farm group.

	Lowland farms (Thurgau)		Mountain farms (Grisons)	
	Collaborating	Non-collaborating	Collaborating	Non-collaborating
GENERAL CHARACTERISTICS				
Conventional/Organic farming	4/0	4/0	1/3	4/0
Elevation (m a.s.l.)	471 ± 66	533 ± 40	1000 ± 285	885 ± 173
Percentage of area with slope > 18%	0 ± 2	2.5 ± 3	68 ± 10	50 ± 23
FARM AREA				
Total agricultural area (ha)	36.5 ± 9.6	43.3 ± 11.0	38.7 ± 9.6	36.4 ± 9.4
of which				
Grassland (ha)	24.6 ± 1.5	24.6 ± 14.0	38.2 ± 11.2	32.9 ± 7.6
Maize for whole-crop forage (ha)	3.8 ± 2.6	4.3 ± 2.8	0 ± 1.0	2.1 ± 2.8
Fodder beets (ha)	—	0 ± 0.15	—	—
Cereals (ha)	3.1 ± 3.1	5.3 ± 2.4	0.5 ± 1.4	—
Grain maize (ha)	—	1.0 ± 1.6	—	—
Oil seeds (ha)	0 ± 0.5	0 ± 0.5	—	—
Sugar beets and potatoes (ha)	3.8 ± 2.3	0 ± 0.9	—	—
Orchards and other crops (ha)	0 ± 2.8	0.3 ± 2.2	—	—
DAIRY ENTERPRISE				
Livestock units (LU)	66.0 ± 9.2	90.1 ± 30.0	26.8 ± 2.5	44.9 ± 10.9
of which dairy cows	58.8 ± 8.3	70.8 ± 25.4	0 ± 1.13	33.0 ± 9.2
LU per ha UAA dairy enterprise	2.33 ± 0.33	2.24 ± 0.45	0.74 ± 0.16	1.11 ± 0.42
FPCM sold per dairy cow (kg/a)	8844 ± 818	8608 ± 900	—	7682 ± 1060
Concentrate per cow (kg/a)	922 ± 582	1931 ± 532	—	955 ± 466
of which home-grown (kg/a)	102 ± 164	273 ± 310	—	0 ± 0
Other purchased feed per cow				
Grass, silage or hay (kg DM/a)	167 ± 454	0 ± 17	—	111 ± 169
Whole-crop maize (kg DM/a)	294 ± 450	54 ± 216	—	—
Beets or potatoes (kg DM/a)	408 ± 183	33 ± 82	—	—

DM, dry matter; FPCM, fat- and protein-corrected milk; m a.s.l., meters above sea level; UAA, usable agricultural area.

most suitable to account for interactions between cash-crop and dairy enterprises (Marton et al., 2016a). All emissions related to application of manure to cash crops were attributed to the dairy enterprise, while emissions that a mineral fertilizer would have caused, as well as emissions from producing it, were credited to the dairy system. The amounts of nutrients replaced by manure applied within the cash-crop enterprise were calculated based on crop requirements and the nutrient availabilities of manure and mineral fertilizers. The amount of nitrogen (N) replaced

was calculated based on the total ammonium N (TAN) in the applied manure (Flisch et al., 2009) and the ammonia-loss rates of manure and the mineral fertilizer replaced (Hutchings et al., 2009, 2013). When more N was applied to a crop than its theoretical N requirements, only the amount of N required minus the amount of N provided by other fertilizers was credited (Equation 1). We assumed that manure replaced ammonium nitrate, the mineral N fertilizer most commonly used on farms in our sample.

$$N_{\min_sub} = \text{Min} \left(\frac{TAN_{\text{appl}} \times (1 - r_{\text{org}})}{1 - r_{\min_sub}}; \frac{N_{\text{need}} - N_{\min_appl} \times (1 - r_{\min_appl})}{1 - r_{\min_sub}} \right) \quad (1)$$

N_{\min_appl} , Amount of N from mineral fertilizers applied to crops (in kg).

N_{\min_sub} , Amount of N from mineral fertilizers replaced with manure (in kg).

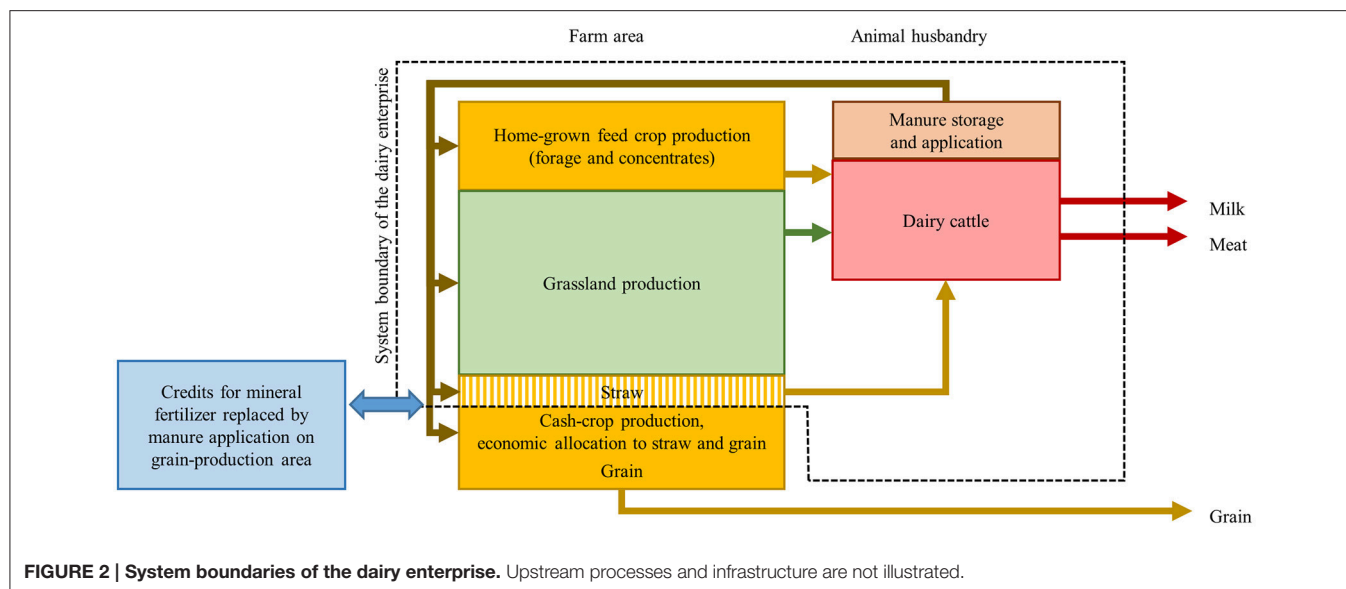
N_{need} , Amount of N required by the crop (in kg).

r_{\min_appl} , Ammonia loss rate of mineral fertilizers applied to crops.

r_{\min_sub} , Ammonia loss rate of mineral fertilizers replaced with manure.

r_{org} , Ammonia loss rate of manure applied to crops.

TAN_{appl} , Total ammonium N in manure applied to crops (in kg).



For phosphorus (P) and potassium (K), the amount of each contained in manure was calculated to replace the same amount from mineral fertilizers, as long as it did not exceed plant requirements. If the latter was the case, only the amount of nutrients needed by the plant and not covered by other fertilizers was credited. The mineral fertilizers assumed to be replaced were triple-superphosphate and potassium chloride (KCl), respectively.

Farming systems are multifunctional, but their main function is to supply food. Therefore, the collaborative and non-collaborative dairy production systems were compared based on their main output, using the functional unit of 1 kg of FPCM. System boundaries were defined in the same way as for the assessment of the impact per hectare of UAA, i.e., considering all upstream processes and activities on the farm, up to the farm gate. Milk production is a multi-output process, and besides the manure that can be exported from the dairy enterprise (accounted for as described above), meat is also produced from culled animals and surplus calves. We followed the guidelines of the International Dairy Federation, using physical causality to allocate environmental impacts to milk and meat (International Dairy Federation (IDF), 2015).

Life Cycle Inventory

The farmers provided the main data used to calculate the LCI, which included data about yields, animal numbers, purchased inputs such as feed or energy carriers, housing infrastructure and manure management, machinery, and detailed information about field-management practices such as date, type, quantity applied, and application method for each fertilization or crop-protection event. The LCI itself was then calculated with a tool developed for the CANTOGETHER project (CANCalc, Teuscher et al., 2014). In the present study, the LCI consisted of an inventory of farm inputs, linked to upstream processes from ecoinvent v2.2 (ecoinvent Centre, 2010) and the Swiss

agricultural LCA (SALCA, Nemecek et al., 2010) database, and farm outputs including direct emissions. The following tools and models were used to calculate direct emissions:

- (1) A dataset of organic and mineral fertilizers, with their nutrient contents and availability to plants, based on data from Flisch et al. (2009), Nemecek and Kägi (2007) and fertilizer producers.
- (2) A tool developed by project partner SP Technical Research Institute of Sweden (formerly SIK) to predict emissions from enteric fermentation, manure management and application (Berglund and Cederberg, 2014).
- (3) The SPACSYS (soil-plant-atmosphere continuum system) model, version 5.1 (Wu et al., 2015), to predict N leaching and runoff, and nitrous oxide (N₂O) emissions.
- (4) The Universal Soil Loss Equation (Renard et al., 2011) to predict soil erosion.
- (5) Elements of the SALCA to predict heavy-metal entry into soil and ammonia emissions from mineral fertilizers.

The LCI was calculated for the entire farm and for its individual farm enterprises, according to the system boundaries defined. Inputs and emissions were grouped into 12 categories: buildings and equipment, machinery, energy carriers, fertilizers and field emissions, pesticides, purchased seeds, purchased concentrate, purchased roughage, purchased animals, animal husbandry, other inputs, and summer pasture. The last category is special, since it comprises all inputs and emissions that occur during the summer-pasture phase, without distinguishing their exact sources. These emissions are mainly caused by the animals, either due to enteric fermentation (corresponding to the category “animal husbandry”) or due to excretion directly on the area (corresponding to the category “fertilizers and field emissions”).

For the credits related to manure application within the cash-crop enterprise, two LCI were calculated, one with manure application, and one in which the mineral fertilizers that had

been replaced were applied instead of manure (Equation 1). This was necessary because direct emissions linked to N fertilization, such as nitrate leaching into water or ammonia emissions to the air, are influenced by the type of N source applied. For example, manure has higher ammonia emissions than ammonium nitrate (Hutchings et al., 2009, 2013). If the inventory with manure application had higher direct emissions, the difference was attributed to the dairy enterprise, but if it had lower direct emissions, the difference was credited to the dairy enterprise.

Life Cycle Impact Assessment

We considered impact categories related to three aspects: (1) emissions into the ecosphere, i.e., into the air, soil, or water, (2) land use, and (3) resource use. The impact categories related to emissions into the ecosphere are linked to specific environmental challenges, such as climate change, and thus cover rather narrow topics. Impact categories related to land use are linked to the scarcity of land as a resource, but also to the impact that use of this land could have on biodiversity. The resource-related impact categories, as used in this study, are indicators of both depletion of scarce resources and efficiency of production systems. From these three impact category groups, the following categories were assessed:

- Categories related to impacts caused by emissions into the ecosphere
 - Acidification (EDIP2003, Hauschild and Potting, 2005).
 - Ecotoxicity, terrestrial (CML2001, Guinée et al., 2001).
 - Eutrophication due to N (EDIP2003, Hauschild and Potting, 2005).
 - Eutrophication due to P (EDIP2003, Hauschild and Potting, 2005).
 - Global warming potential over 100 years (IPCC2013, Myhre et al., 2013).
 - Ozone depletion (EDIP2003, Hauschild and Potting, 2005), with the addition of the ozone depletion potential of N₂O according to Ravishankara et al. (2009).
- Categories related to land use
 - Deforestation (Frischknecht et al., 2007a).
 - Land competition (Frischknecht et al., 2007a), excluding alpine summer-pasture land use.
 - Alpine summer-pasture land use.
 - Biodiversity on the farm area (Jeanneret et al., 2014).
- Categories related to resource use
 - Non-renewable energy demand, fossil and nuclear (Frischknecht et al., 2007a).
 - Resource use, P extraction (based on elementary flow from ecoinvent 2.2, Frischknecht et al., 2007b).
 - Resource use, K extraction (based on elementary flow from ecoinvent 2.2, Frischknecht et al., 2007b).
 - Water use (Frischknecht et al., 2007a).

The impact category “ozone depletion potential” was adapted for this study, since currently available life cycle impact assessment methods do not consider the ozone depletion potential of N₂O, although it is currently considered the most important

ozone-layer-depleting substance (UNEP, 2013). Ravishankara et al. (2009) were the first to publish an ozone depletion potential for N₂O: 0.017 kg CFC-11 equivalents per kg N₂O. This ozone depletion potential is valid at present, but future change in gas composition in the stratosphere, namely chlorine, CO₂ and CH₄, may increase the ozone depletion potential of N₂O (Revell et al., 2015). We therefore considered the factor of 0.017 as robust enough to be used in our context. For comparison of results with and without consideration of N₂O, see Supplementary Figure 1.

The impact category of land competition was also adapted for the purpose of the present study. Alpine summer-pasture land use was treated separately, since it does not compete with other anthropogenic land-use types. The area is not suitable for other agricultural practices and is too remote for other land-use purposes such as urban or industrial use. It does, however, compete with the natural vegetation that would be found on these areas if they were not used as summer pastures. In Europe, alpine summer-pasture areas have declined, and if abandoned, shrubs and forests encroach on them (Anthelme et al., 2001). Although LCA studies usually recommend minimizing land use, other research results indicate that conservation and use of summer pasture is beneficial, especially for biodiversity (Pornaro et al., 2013; Koch et al., 2015). In the present study, more alpine summer-pasture land use was thus considered as beneficial. Similar to land competition, alpine summer-pasture land use is expressed as the area occupied multiplied by the duration of the occupation (square meter years—m²a).

The different impact assessment methods were applied for both functional units, per hectare of UAA and per kg of FPCM, except for biodiversity. Compared to the other LCIA methods used, the method to assess biodiversity is a special case. It relies on estimates of effects of various agricultural land use types (i.e., arable crops, grasslands, semi-natural habitats) and agricultural practices (e.g., plowing, pesticide application, date of first cut in grasslands) on local biodiversity. The LCI provided the data required to apply the biodiversity model and estimate scores for overall species diversity. The model considers the suitability of land use types and the severity of agricultural practices on 11 indicator species groups related to farmland. From the individual scores for each species group, the overall species diversity score is calculated. High overall species diversity scores indicate that a system is beneficial for biodiversity (Jeanneret et al., 2014). Because the method focuses on a farm's agricultural area, its system boundaries exclude upstream processes. Also, since it uses scores instead of quantitative units, these scores cannot be attributed to single products; thus, biodiversity was assessed only per hectare of UAA.

Workload Assessment

Workload related to dairy production was calculated for each farm with the workload budgeting tool ART-AV 2014 (Stark et al., 2014). The tool considers the crop and grassland fields on the farm, and animal numbers of several livestock categories, such as calves, heifers, dairy cows, fattening cattle and pigs. It also considers economies of scale, for instance assuming lower workloads per hectare for larger areas of the same crop produced on a given farm. Using the same allocation rules as for the LCA, we calculated the workload for the dairy enterprise, expressed as

hours per hectare of UAA, as well as the hours needed to produce 1 kg of FPCM.

Statistical Tests and Sensitivity Analyses

Differences between collaborative and non-collaborative dairy production systems and between collaborative and non-collaborative farms within each region for the environmental impact categories and workload indicators were tested for significance with a one-sided Mann-Whitney *U*-test. Comparison of the dairy production systems considered two groups with 16 data points each, a number of observations that is large enough to provide meaningful results. Comparison of the farms within a given region, however, had only two groups with 4 data points each. A one-sided test with such a small sample size will result in a $p < 0.05$ only if one group contains the first four ranks or the first three and the fifth rank. Since these cases are rare, we therefore also considered tendencies, defined as $p \leq 0.10$, when comparing farms within a given region. A value of $p = 0.10$ would correspond to a situation in which the three best-performing farms are in one group and the worst-performing farm is in the other group, i.e., one group contains ranks 1, 2, 3, and 7, and the other 4, 5, 6, and 8.

Given the sample size, the sample farms do not necessarily represent the entire population of collaborative and non-collaborative dairy farms in Switzerland. For example, while more than 50% of all farms in Grisons are organic (Swiss Federal Statistical Office, 2016), none of the non-collaborating farms in the Grisons sample was organic. Two of them, however, used no mineral fertilizers or pesticides, and thus differed from organic farms only in the type of concentrate purchased. We therefore performed a sensitivity analysis in the comparison of farms, in which these two farms were virtually converted into organic farms by replacing purchased conventional concentrate with organic concentrate. Another sensitivity analysis was performed for credits for mineral fertilizer replaced by manure applied to cash crops. In it, we applied an allocation procedure based on a cut-off principle: all emissions from manure storage were allocated to the dairy enterprise, while those from manure application outside of the dairy enterprise were allocated to the cash-crop enterprise. This allocation procedure corresponds to that in ecoinvent v2.2 (Nemecek and Kägi, 2007) and is also recommended in the IDF's current LCA guidelines, while in the previous version the IDF recommended crediting mineral fertilizers that are replaced (International Dairy Federation (IDF), 2010, 2015).

RESULTS

Environmental Impacts

On lowland farms, environmental impacts per hectare of UAA of collaborating and non-collaborating farms did not differ significantly, not even in tendency (Figures 3–5). In the mountains, collaborating farms had significantly lower environmental impacts for terrestrial ecotoxicity, eutrophication due to N, deforestation, land competition, non-renewable energy demand, P and K resource use, and a tendency for lower emissions for acidification and water use. In addition, there was a tendency for higher alpine summer-pasture land use and

higher biodiversity on collaborative mountain farms than on non-collaborative mountain farms.

In both regions, variability in environmental impacts within a group of farms was often higher than differences between the groups. This was most prominent for ecotoxicity of collaborating lowland farms (Figure 3B), one of which had much higher emissions than non-collaborating lowland farms. The high ecotoxicity on this farm was due to a relatively large amount of potatoes in the feed ration, and potato production was linked to high pesticide use. Two further impact categories with high variability were P and K resource use (Figures 5B,C), which both depended highly on the fertilizer strategy and the crops grown on individual farms. Negative impacts (i.e., a positive effect on the environment) resulted from credits due to manure applied to cash crops. Two of the collaborating lowland farms grew sugar beets, a crop with high K demand. Application of manure on this crop led to high credits, which in one case were much higher than the K resource use linked to the inputs of the farm's dairy enterprise. In contrast, one collaborating lowland farm had relatively high K resource use, since it was the only lowland farm that applied KCl to grassland. The same was true for one non-collaborating mountain farm, which applied KCl to both grassland and maize.

Per kg of FPCM, collaborative production caused lower or equal environmental impacts compared to non-collaborative production (Figure 6), except for alpine summer-pasture land use, for which collaborative production used more area. Differences were significant for terrestrial ecotoxicity, eutrophication due to P, ozone depletion, deforestation, alpine summer-pasture land use, non-renewable energy demand, P resource use, and water use. As for individual farms, variability among farm combinations was high. For some farm combinations, the credits attributed to the dairy enterprise due to manure applied to cash crops led to negative K resource use, especially when two farms were combined that both had a negative K resource use.

Sensitivity Analyses

In the first sensitivity analysis (two non-collaborating mountain farms virtually converted to organic farms), impacts for terrestrial ecotoxicity, deforestation, and P and K resource use decreased (Table 2). Nonetheless, non-collaborating mountain farms still had significantly higher impacts for eutrophication due to N, land competition, non-renewable energy demand, and a tendency for higher impacts for acidification, ecotoxicity, and water use. In contrast, deforestation was no longer significantly higher on non-collaborating farms. For P and K resource use, the formerly significant differences were reduced, but a tendency for higher use of these elements on non-collaborating mountain farms remained. Per kg of FPCM, the conversion had no substantial influences. Differences between collaborative and non-collaborative production remained significant for terrestrial ecotoxicity, eutrophication due to P, ozone depletion, deforestation, alpine summer-pasture land use, non-renewable energy demand, P resource use, and water use.

The second sensitivity analysis concerned allocation of emissions from manure that was applied outside of the dairy enterprise, i.e., on cash crops (credits or cut-off approach). The

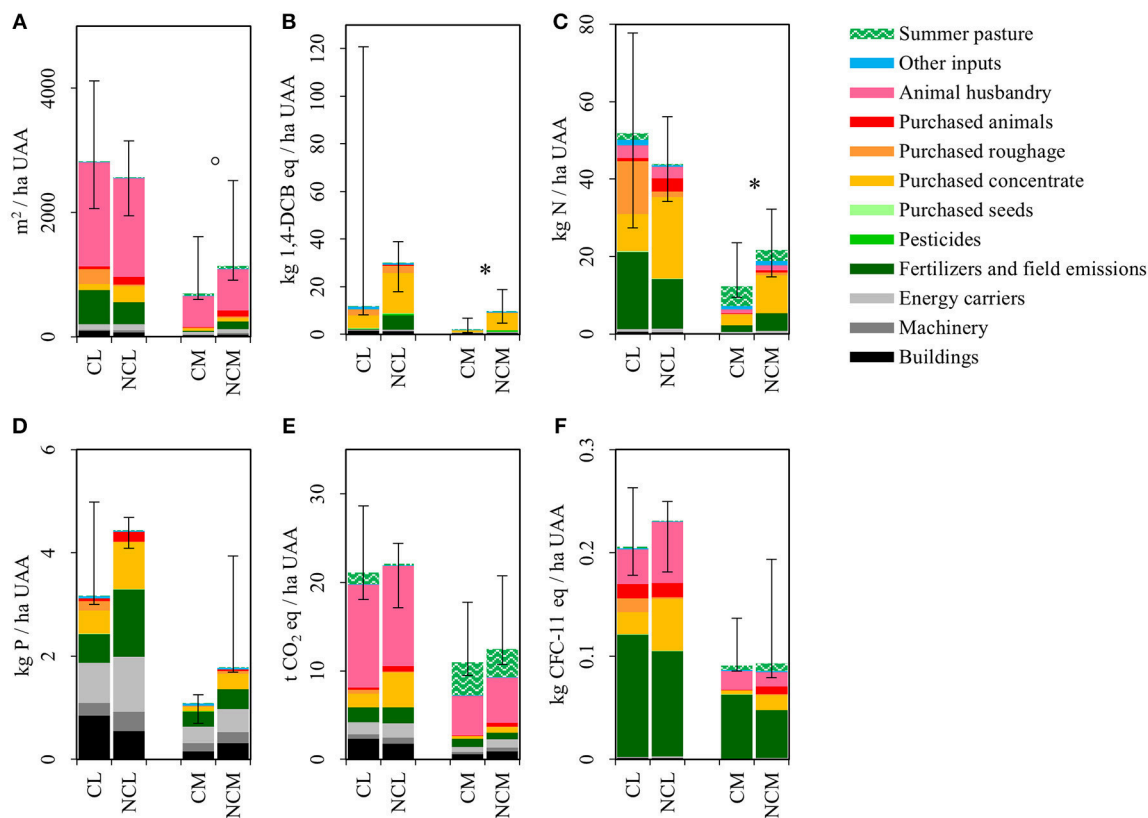


FIGURE 3 | Environmental impact per hectare usable agricultural area (ha UAA) for impact categories related to emissions into the ecosphere:

(A) acidification, (B) terrestrial ecotoxicity, (C) eutrophication due to N, (D) eutrophication due to P, (E) global warming potential, (F) ozone depletion potential. Bars indicate the median performance of each farm group (CL, collaborating lowland farms; NCL, non-collaborating lowland farms; CM, collaborating mountain farms; NCM, non-collaborating mountain farms) and sources of impacts. Whiskers indicate the minimum and maximum impact within each group, *indicate significant differences between farm groups within a given region ($p < 0.05$), ° indicate tendencies ($p \leq 0.1$).

choice of allocation method influenced mainly the results for P and K resource use (Table 3). For farms that applied manure outside of the dairy enterprise, P and K resource use of the dairy enterprise was higher under the cut-off approach. However, the choice of allocation method affected only absolute results and not differences between farms within a given region. In the lowlands, differences between collaborating and non-collaborating farms remained insignificant. In the mountains, collaborating farms still had significantly lower P and K resource use per ha UAA than non-collaborating farms. Per kg of FPCM, on the other hand, the choice of allocation method influenced differences in K resource use: under the cut-off approach, differences between collaborative and non-collaborative production were no longer significant. For the other impact categories, the choice of allocation method did not influence the significance of differences between collaborative and non-collaborative production.

Workload

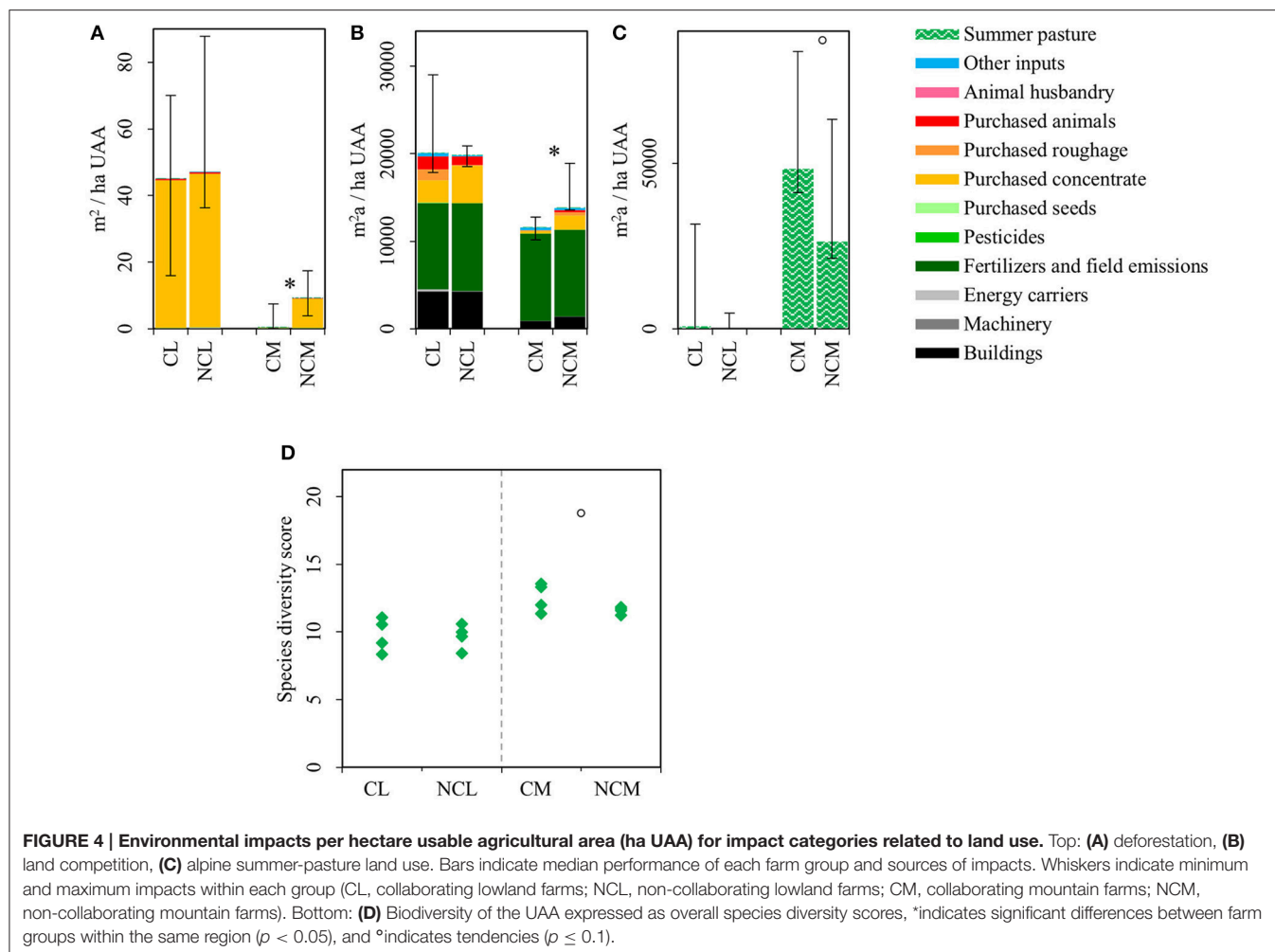
In the lowlands, collaborating farms had slightly higher (but not significantly so) median workload per hectare of UAA than non-collaborating farms. In the mountains, however, collaborating farms had significantly lower workload per hectare of UAA

(median of 64 h/ha UAA) than non-collaborating farms (median of 134 h/ha UAA). Workload per kg of FPCM was significantly lower for collaborating farms (Figure 7).

DISCUSSION

Effects of Collaboration on Environmental Performance of Lowland Farms

We hypothesized that collaborative production would lead to intensification of lowland farms due to outsourcing of the less-intensive heifer rearing activity and an increase in environmental impacts per hectare of UAA. Overall, this hypothesis was not confirmed. There were no significant differences between the collaborating and non-collaborating farms. Variability of the results within each group was higher than differences between the two groups. Concentrate use explained most of the differences in environmental impacts among lowland farms. For most impact categories, differences between individual farms were strongly influenced by the amount of concentrate purchased, although concentrate was not always the main contributor to environmental impacts in absolute terms. For example, enteric fermentation from animals (animal husbandry) contributed most



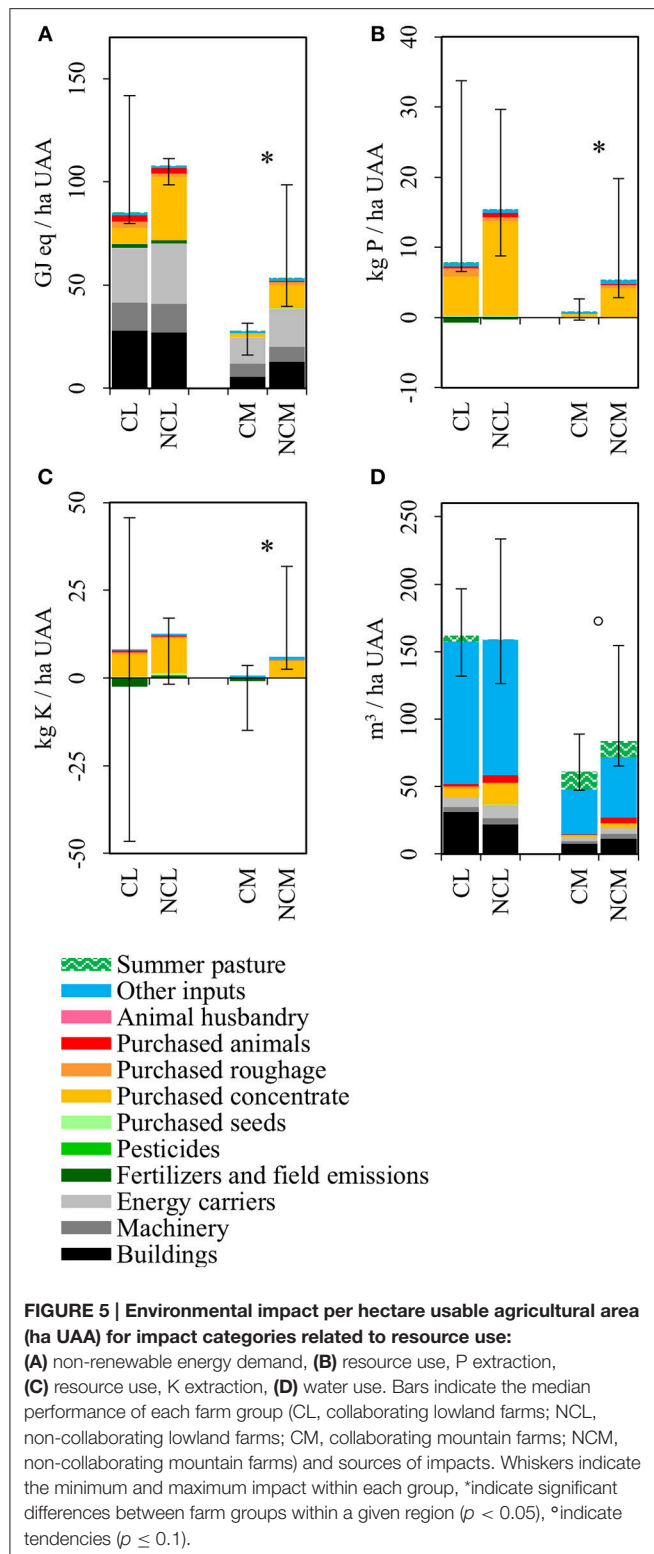
to global warming potential, but it varied little among farms. Concentrate contributed only moderately to global warming potential but had high variability, making it responsible for differences among farms.

Livestock density (expressed as livestock units/ha UAA) was similar for both collaborating and non-collaborating farms, but most animals on collaborating farms were dairy cows, which usually depend more on external inputs such as concentrate. However, concentrate use per hectare was not higher on collaborating than on non-collaborating farms. Per cow, three of the collaborating farms used less concentrate than any of the non-collaborating farms, while achieving similar milk yields. These farms were able to produce more milk with home-grown feed, which indicated higher quality or more efficient use of home-grown forages. We presume that this was an effect of farm specialization, which reduces complexity of farm management, often increasing efficiency (Kingwell, 2011). More efficient use of farm resources may decrease environmental impacts. On the other hand, one of the four collaborating lowland farms used more concentrate per cow than any other farm in the sample and it was also the farm with the highest milk yield per cow. Consequently, it had the highest impacts per hectare of UAA

for most of the impact categories studied. Our findings indicate that collaboration does not necessarily lead to intensification on lowland farms. It depends more on other farm strategy decisions whether a farm intensifies or not.

Effects of Collaboration on Environmental Performance of Mountain Farms

For mountain farms, we hypothesized that due to reduced intensity, collaborating farms would have lower environmental impacts per hectare of UAA than non-collaborating farms. Our results supported this hypothesis. But a lower intensity was not the only reason for the better environmental performance of collaborating farms; organic farming led to additional benefits. Organic production is relatively common for collaborating mountain farms, since a special exception in the directive for organic production allows them to purchase animals from conventional farms in the case of contract rearing (Bio Suisse, 2016). Since collaborating farms rely on relatively low amounts of external inputs, conversion to an organic farm has relatively low cost. In addition, organic farms receive higher subsidies, making organic farming more attractive.

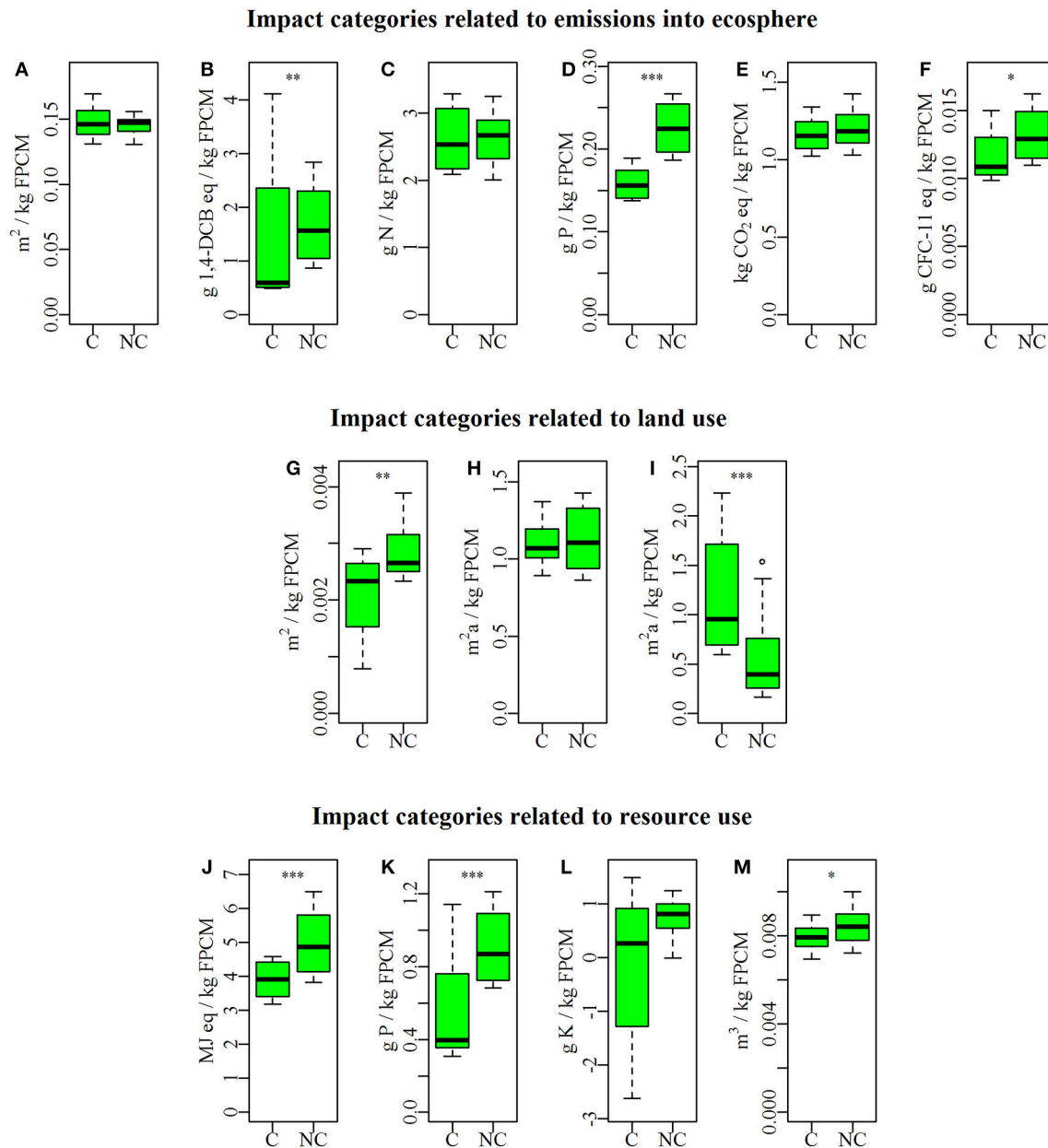


On the other hand, organic farming was also a source of uncertainty in the present study. This farming practice was well-represented in the sample of collaborating mountain farms but underrepresented in the sample of non-collaborating

mountain farms. The sensitivity analysis showed that organic farming practices influenced impacts of mountain farms, especially those related to mineral fertilization (P and K resource use), pesticide application (terrestrial ecotoxicity) and concentrate use (deforestation). Accordingly, for these impact categories, net differences between the population of collaborating and non-collaborating mountain farms in Switzerland may be smaller than apparent from the sample of farms investigated. For deforestation, virtual conversion of the two conventional non-collaborating farms into organic farms reduced impact to the point that non-collaborating farms no longer differed from collaborating farms. This was due to only one feed ingredient: soybean meal. Soybean meal from Brazil is considered to be the main source for deforestation in conventional concentrate. The organic concentrate used in this study from the SALCA database (Nemecek et al., 2010) contained no soybean meal from deforested areas. In contrast, the conventional concentrate included soybean meal from the global market and thus also from Brazil. Consequently, use of organic concentrate led to much lower deforestation. However, this result is valid only as long as indirect land-use change is excluded from the assessment (Meyfroidt et al., 2013), since land competition itself did not decrease in the scenario with more organic farms.

Alpine Summer-Pastures

Mountain farms generally practice more alpine summer-grazing, but due to the steep slopes and relatively poor feed quality on these pastures and the higher feed requirements of today's dairy cows, the traditional practice of summer pasture is becoming less attractive to dairy farmers (Penati et al., 2011). It is therefore not surprising that collaborating mountain farms used more summer pastures than non-collaborating mountain farms, since it is still convenient to use these pastures for less demanding animals. In this study, we considered greater use of these areas as a benefit, especially for biodiversity. Still, the effective biodiversity value of summer pasture also depends on its management. The biodiversity assessment method used in the present study (Jeanneret et al., 2014) considers management practices at the farm level, but so far it is not applicable to summer pasture. Therefore, we can discuss effects of possible changes in summer-pasture management only qualitatively. Alpine summer-pasture land use by non-collaborating and collaborating mountain farms differs in the type of animals they send to summer pasture, the former sending both dairy cows and heifers, the latter sending almost only heifers. Since cows and heifers may differ in their grazing patterns, the question arises whether dairy cows or heifers are best suited to maintain optimal summer-pasture vegetation. In the study of Homburger et al. (2015), dairy cows showed different grazing and land-use patterns than suckler beef cows. Dairy cows tended to avoid steeper slopes more than suckler beef cows did. During the night, dairy cows were housed either in a shed or on a small paddock near the farm buildings where animals were milked, while suckler beef cows were kept on the same paddocks as during the day. Homburger et al. (2015) did not study heifers, but we expect that they behave more like suckler beef cows than dairy cows. Since suckler beef cows and heifers are not milked, they can be sent to summer pastures without



the need to keep them close to farm buildings. Because they weigh less, heifers are also more suited to steeper slopes and cause less treading damage than heavier animals (Greenwood and McKenzie, 2001). We therefore expect that heifers are better suited to management that optimizes and maintains high biodiversity on summer pasture; thus, collaborative mountain farms have not only greater, but potentially more beneficial, use of summer pastures.

Effect of Collaboration on Environmental Impacts per Kg of Fat- and Protein-Corrected Milk

We hypothesized that collaborative production would reduce environmental impacts per kg of FPCM. This was supported by the LCA results and confirmed the tendencies already observed at the farm level. Our results were also consistent with those

TABLE 2 | Sensitivity analysis: conversion of two non-collaborative mountain farms into organic farms (50% organic scenario) compared to the original scenario, in which all non-collaborative mountain farms were conventional farms (main scenario) for the impact categories affected the most by this change (terrestrial ecotoxicity, deforestation, P resource use, K resource use).

	Terr. ecotoxicity (kg 1,4-DCB eq)	Deforestation (m ²)	P resource use (kg P)	K resource use (kg K)
MEDIAN NON-COLLABORATING MOUNTAIN FARMS (PER HA UAA DAIRY ENTERPRISE)				
Main scenario	9.58	9.21	5.31	6.06
50% organic scenario	7.94	5.00	3.79	3.88
Change (%)	-17.1	-45.7	-28.5	-36.0
MEDIAN NON-COLLABORATIVE DAIRY PRODUCTION SYSTEM, MOUNTAIN, AND LOWLAND FARMS COMBINED (PER KG OF FPCM)				
Main scenario	1.57×10^{-3}	2.66×10^{-3}	8.71×10^{-4}	8.05×10^{-4}
50% organic scenario	1.57×10^{-3}	2.57×10^{-3}	8.57×10^{-4}	7.73×10^{-4}
Change (%)	-0.09	-3.30	-1.60	-3.95

UAA, usable agricultural area; FPCM, fat- and protein-corrected milk.

of a previous assessment based only on simulated farms, for which collaborative production was calculated to have lower non-renewable energy demand and lower resource use both for P and K (Marton et al., 2016b). Compared to those of the simulated farms, the differences found in the present assessment of real farms were larger. We assume that the real farms not only benefited from effects of the principle of comparative advantage observed under farm simulations but were also able to improve the system further by increasing efficiency gains via specialization, as observed for the collaborating lowland farms.

Still, our assessment is based on a small and possibly biased sample. We identified two possible causes for bias. First, none of the non-collaborating mountain farms was organic. At the farm level, virtually converting half of them into organic farms reduced certain impacts of the group, but not enough to influence results per kg of FPCM. This was because mountain farms in the non-collaborative system contributed only 11% of total milk production, due to two reasons: the ratio of lowland to mountain land use was set to 3.5:1 (the median ratio in the collaborative system), and mountain farms produced less milk per ha than lowland farms. The second possible bias came from sampling only dairy farms that offered apprenticeship positions. To hire apprentices, farmers need to have additional training. We assume that most farmers who are willing to help train the next generation are more motivated to keep themselves updated about agricultural developments. If so, their farms may have above-average environmental performance. In any case, direct comparison with other LCA studies is always challenging, since system boundaries and allocation procedures vary, as do the methods used to calculate direct emissions (de Vries and de Boer, 2010). Therefore, we have no evidence for the magnitude of bias in the samples. If bias does exist, it is reasonable to assume that it affects all samples equally and thus does not change the conclusions drawn.

Effects of Collaboration on Workload

Our hypothesis that collaboration would reduce workload was partially confirmed. The reduction in workload on collaborating mountain farms was the most distinct, since heifers require less labor to care for than dairy cows. On lowland farms, the

most important influences on workload were economies of scale, which were considered by the workload estimation tool. By outsourcing heifers, collaborating lowland farms increased the number of their dairy cows, obtaining economies of scale. However, non-collaborating dairy farms from our sample tended to be larger. Consequently, they kept more dairy cows than collaborating dairy farms and thus could also profit from economies of scale. The effect of larger size was at least as beneficial to non-collaborating farms as the effect of having fewer animal categories was to collaborating farms. Our results confirm those of Regan et al. (2016), who analyzed workload based on self-declaration by farmers in the same farm sample. Their approach considered all farming activities combined (i.e., dairy and cash-crop enterprises), since it was not possible for farmers to indicate workload for each enterprise on the farm, but this likely had no major influence on the results.

Combining workloads of farms from the two regions, collaboration decreased workload per kg of FPCM. This was caused mainly by lower workload on mountain farms under collaboration, but also by higher output of milk per ha UAA on lowland farms under collaboration. Like environmental impacts, higher work efficiency in the collaborative production system was assumed to be a combination of benefits from focusing on an activity with comparative advantage and from specialization.

Contribution of the System to Preservation of High Nature Value Farmland in the Mountain Region

The contract rearing system increased eco- and work-efficiency of dairy production in our sample, but the question remains if it also helps to preserve high-nature-value farmland in the mountains. High nature value farmland in the mountains is threatened by two developments: intensification and abandonment. Both result mostly from the lower economic viability of traditional mountain farming systems (Strohbach et al., 2015). Regarding the intensification issue, replacing dairy cows with heifers would maintain agricultural use and, at the same time, reduce intensity of farmland use. Land on collaborating mountain farms was managed less intensively than that on non-collaborating mountain dairy farms, since the

TABLE 3 | Sensitivity analysis: cut-off approach for manure applied outside of the dairy enterprise, in which emissions from manure application lay outside of the scope of the dairy enterprise (cut-off for manure), compared to the original scenario, in which credits for mineral fertilizers replaced by manure were applied (main scenario) for the impact categories affected the most by this change (P resource use, K resource use).

	P resource use (kg P)	K resource use (kg K)
MEDIAN COLLABORATING MOUNTAIN FARMS (PER HA UAA DAIRY ENTERPRISE)		
Main scenario	0.66	-0.09
Cut-off for manure	1.48	1.44
Change	+124.8%	n/a
MEDIAN NON-COLLABORATING MOUNTAIN FARMS (PER HA UAA DAIRY ENTERPRISE)		
Main scenario	5.31	6.06
Cut-off for manure	5.31	6.06
Change (%)	0.00	0.00
MEDIAN COLLABORATING LOWLAND FARMS (PER HA UAA DAIRY ENTERPRISE)		
Main scenario	7.04	5.73
Cut-off for manure	7.81	9.81
Change (%)	+11.0	+71.1
MEDIAN NON-COLLABORATING LOWLAND FARMS (PER HA UAA DAIRY ENTERPRISE)		
Main scenario	15.04	12.56
Cut-off for manure	15.36	13.90
Change (%)	+2.1	+10.7
MEDIAN COLLABORATIVE DAIRY PRODUCTION SYSTEM, MOUNTAIN, AND LOWLAND FARMS COMBINED (PER KG FPCM)		
Main scenario	3.96×10^{-4}	2.63×10^{-4}
Conversion of two conventional farms into organic	4.21×10^{-4}	5.12×10^{-4}
Change (%)	+6.5	+94.3
MEDIAN NON-COLLABORATIVE DAIRY PRODUCTION SYSTEM, MOUNTAIN, AND LOWLAND FARMS COMBINED (PER KG FPCM)		
Main scenario	8.71×10^{-4}	8.05×10^{-4}
Conversion of two conventional farms into organic	8.82×10^{-4}	9.77×10^{-4}
Change (%)	+1.3	+21.4

UAA, usable agricultural area; FPCM, fat- and protein-corrected milk.

former used fewer external inputs and had a lower stocking rate. This low-input farming system thus corresponds more to the relatively low intensity of traditional farming systems in the mountain area, which shaped the mountain farming area's high nature value.

An important constraint, however, is that contract rearing alone might not be sufficient to prevent farmers from abandoning their land, because it would reduce not only the workload of mountain farms but also the income. Therefore, compensating the income loss with off-farm labor would be necessary to make the system viable (Marton et al., 2016b). In Switzerland, off-farm labor usually pays better than on-farm labor (Hoop and Schmid, 2014; Swiss Federal Statistical Office, 2015), but jobs

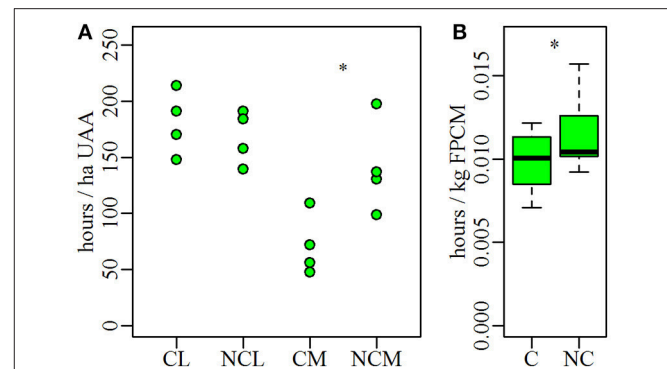


FIGURE 7 | Comparison of workload (A) per hectare usable agricultural area (ha UAA) of the dairy enterprise on collaborating lowland (CL, $n = 4$), non-collaborating lowland (NCL, $n = 4$), collaborating mountain (CM, $n = 4$), and non-collaborating mountain (NCM, $n = 4$) farms; and (B) per kg fat- and protein-corrected milk (FPCM) in collaborative (C, $n = 16$) and non-collaborative (NC, $n = 16$) dairy production systems. Whiskers indicate 1.5 times the standard deviation, and *indicates significant differences between farm groups ($p < 0.05$).

are not always available in the remote mountain regions. The sampled contract rearing mountain farms effectively gained a large percentage of their family income from off-farm labor. Furthermore, and in contrast to results from simulated farms, these farms also managed to achieve incomes that were at the same levels as those of non-collaborating mountain dairy farms (Regan et al., 2016). Collaboration decreased the risk of land abandonment due to economic reasons for the farms in the sample, since it provided farmers with a reasonable income.

The contract rearing system can keep mountain areas in production and may even increase the high nature value of mountain farmland through its less intensive management. Since high-nature-value farming provides provisioning, regulating and cultural ecosystem services (Plieninger and Bieling, 2013), the system is also expected to contribute to the important role of mountainous regions in providing such services.

From the Cantonal to National Level

The sample farms in this study were located in Thurgau and Grisons, since the system is most popular in these two cantons, but farms in other cantons also practice contract rearing. The contract rearing mountain farms in the sample collaborated not only with lowland farms from Thurgau, but also from Lucerne, Aargau and Zurich. The collaborating lowland farms in the sample cooperated either with farms from Grisons or St. Gall. In most cantons, agricultural extension services offer a brokering service for farmers to facilitate collaboration (Agridea, 2016). Whether results based on data from farms in only two cantons are valid for the entire country depends on the degree to which they represent Switzerland's lowland and mountain zones. Classification of Switzerland into mountain, hill and lowland zones is based on climatic and topographic characteristics (Federal Office for Agriculture, 2016). Production conditions for farms from Thurgau and Grisons should therefore be similar

to those of farms in other lowland and mountain regions, respectively. Furthermore, results from the present study were in line with those from the study with simulated average Swiss farms (Marton et al., 2016b). It is therefore probable that the benefits observed for the sample farms are also valid for farms from other cantons of Switzerland. Still, it is unlikely that the system will be extended to all Swiss mountain dairy farms. Even if all lowland farms outsourced their young stock to mountain farms, the demand for contract rearing would involve only approximately one-third of all mountain dairy farms (Marton et al., 2016b). The resulting reduction in mountain milk production would most likely not affect production of traditional mountain cheese, since farms producing milk designated for cheese production receive an extra price premium (Finger et al., 2015), which acts as an incentive to continue dairy production. Farms that produce milk designated for other purposes and not marketed as a mountain product are more likely to abandon milk production.

CONCLUSION

Using the example of a contract rearing system between farms in a favorable region and a region with natural constraints, we demonstrated how collaboration can help decrease environmental impacts of agricultural production while keeping the areas with natural constraints productive and thus preserve these high nature value areas. We identified two effects responsible for the improvement. The first was associated with comparative advantages, in which each collaboration partner focused on an activity with lower opportunity costs. The collaborative system allowed both mountain and lowland farms to concentrate on the phase within the life of a dairy cow that corresponded best to the resources available on their land. The steeper land and lower energy content of mountain grass can fulfill the needs of young stock, while lowland farms can produce high-quality grass, other forages such as maize, and the concentrate needed to meet the higher energy requirements of lactating dairy cows. The second effect responsible for lower impacts under collaboration was specialization. Specialization can reduce management complexity of farms, and focusing on fewer activities helps to increase the skills necessary to perform them. Limitations of our study included the relatively

small sample size and the potential overrepresentation of more advanced dairy farms. Further, research is needed to test whether the effects also apply to average dairy farms. We are confident, however, that reduction in management complexity will be especially beneficial for more average collaborating farms. Furthermore, we believe that this example of contract rearing involving favorable and less favorable regions could encourage development of other collaborative production systems. It would be of great interest to study the applicability of this principle to other agricultural production systems with a regional division of labor or to other geographic and climatic regions.

AUTHOR CONTRIBUTIONS

SM is the corresponding author and main researcher. GL performed the biodiversity assessment. MC performed some of the LCA calculations. MK is project collaborating partner and supervisor. GG is project leader and co-supervisor. GL, MC, MK, and GG contributed to finalizing the manuscript.

ACKNOWLEDGMENTS

We wish to express our gratitude to the farmers who provided the data for our assessment. We also thank Rémy Teuscher, Peter Koch, Hisko Baas, and Jens Lansche for the development of the CANTOGETHER LCA tools, and Lianhai Wu for his support with SPACSYS. We would also like to thank Daniel U. Baumgartner for his support and proofreading of the manuscript, and Thomas Nemecek for his methodological advice during the project. This work was funded under the EU Seventh Framework Program by the CANTOGETHER project (no. 289328): Crops and ANimals TOGETHER. The views expressed in this work are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fenvs.2016.00074/full#supplementary-material>

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Conflict of Interest Statement: 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.

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Effects of Litter on Seedling Emergence and Seed Persistence of Three Common Species on the Loess Plateau in Northwestern China

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OPEN ACCESS

Edited by:

Maria Tsiafouli,
Aristotle University of Thessaloniki,
Greece

Reviewed by:

Yong Liu,
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 24 July 2016

Accepted: 18 January 2017

Published: 31 January 2017

Citation:

Zhang R, Hu X, Baskin JM,
Baskin CC and Wang Y (2017)
Effects of Litter on Seedling
Emergence and Seed Persistence
of Three Common Species on
the Loess Plateau in Northwestern
China. *Front. Plant Sci.* 8:103.
doi: 10.3389/fpls.2017.00103

Litter accumulation resulting from land use change (enclosure) is one of the key variables influencing seedling recruitment and consequently the regeneration of plant populations and seed persistence in the soil seed bank. A better understanding of the effects of litter on seed germination and seedling emergence is crucial for developing a new set of indicators for grassland ecosystem health and for grassland management policy. We investigated the effects of seed position in litter and amount of litter covering the seed on seedling emergence and seed persistence of three common species on the Loess Plateau in northwestern China. Seed position beneath the litter layer provided a suitable environment for seedling emergence of the three species. A moderate amount of litter (160 g/m²) was beneficial for seedling emergence of the small-seeded species *Stipa bungeana* and *Lespedeza davurica* from seeds from beneath the litter layer. The large-seeded species *Setaria glauca* was more tolerant of a high amount of litter (240 g/m²) than the two small-seeded species. Seed persistence in the soil differed among the three species and also was affected by seed position in litter and amount of litter cover. The proportion of viable seeds of *Stipa bungeana* and *Setaria glauca* on top of the litter layer increased with an increase in amount of litter. Seedling emergence and seed persistence varied significantly among species, amount of litter and seed position in litter. A moderate amount of litter and seeds positioned beneath the litter layer were better for seedling recruitment than for those on top of the litter layer. A high amount of litter was more favorable for persistence of seeds positioned on top of the litter than for those beneath the litter. Our study showed that maintaining litter amount between 80 and 160 g/m² is optimal for *S. bungeana* dominated grassland on the Loess Plateau. We suggest that litter amount can serve as a guide for monitoring and managing grassland ecosystems, as it is an indicator of ecosystem processes that are essential for biodiversity conservation and restoration.

Keywords: grassland ecosystem health, *Lespedeza davurica*, litter, Loess Plateau, seedling emergence, seed persistence, *Setaria glauca*, *Stipa bungeana*

INTRODUCTION

Litter plays an important role in influencing community structure (Grime, 2001) and may affect the establishment of new individuals as a form of post-death, plant–plant interaction (Facelli and Pickett, 1991a). A litter layer acts as a mechanical barrier to seedling emergence, changes physical factors of the environment, such as soil temperature and moisture and light quantity and quality and indirectly modifies the chemical environment by releasing allelochemicals that affect seedling establishment and seedling growth (Facelli and Pickett, 1991a; Holmgren et al., 1997; Ruprecht et al., 2010a). Thus, litter may influence the diversity and dynamics of plant communities, and consequently productivity and health of ecosystem (Fowler, 1986; Gibson and Good, 1987; Facelli and Pickett, 1991b; Quedsted et al., 2005; Ruprecht et al., 2010b; Eckstein et al., 2011).

The effect of litter on seedling emergence and consequently seedling recruitment varies with environmental conditions, litter amount, seed position and seed traits (Hovstad and Ohlson, 2008; Wellstein, 2012; Egawa and Tsuyuzaki, 2013; Loydi et al., 2013). A meta-analysis showed that litter had an overall negative effect on seed germination and seedling establishment in grassland, old field and forest ecosystems (Xiong and Nilsson, 1999), but recent studies indicated that this might not be the case in grassland ecosystems (Quedsted and Eriksson, 2006; Donath and Eckstein, 2008; Loydi et al., 2013). For dry grasslands or grasslands under water-limited conditions, low to medium amounts of litter had a positive effect on seedling emergence and recruitment. However, a high amount of litter will inhibit seed germination and seedling establishment by reducing light quality and quantity beneath the litter layer, by preventing seeds on top of the litter from reaching the soil or by preventing roots of seedlings that germinate on top of the litter layer from reaching the soil surface (Xiong and Nilsson, 1999; Eckstein and Donath, 2005; Donath and Eckstein, 2010; Loydi et al., 2013).

In addition to amount of litter, the responses of plants to litter depend on seed position in/on the litter layer. Seeds can be positioned on top, within or beneath the litter layer depending on whether litter input occurs before, during or after seed rain (Wellstein, 2012). For example, seedling emergence of *Pimpinella saxifraga*, *Leontodon autumnalis*, and *Sanguisorba officinalis* was significantly higher for seeds beneath the litter layer than for those on top of it or on the bare soil (Wellstein, 2012). In contrast, germination of forest understory *Carex* species was lower for seeds beneath the leaf litter than for those on top of the litter layer (Vellend et al., 2000).

The soil seed bank is an important consideration in community ecology, and above-ground vegetation dynamics, and knowledge of seed persistence in the soil plays an important role in the conservation of rare species, in maintenance of plant communities and in restoration of vegetation following disturbances (Van der Valk and Verhoeven, 1988; Hodgson and Grime, 1990; Jones and Esler, 2004; Bossuyt and Honnay, 2008; Michaela and Wolfgang, 2009; Baskin and Baskin, 2014; Long et al., 2015). Seed persistence is affected by many factors, such as seed characteristics, species characteristics and abiotic and biotic conditions in the pre-dispersal and

post-dispersal environments (Long et al., 2015). Kettenring et al. (2006) reported that seed bank development is not only determined by the standing vegetation but also by modification of microenvironments through litter accumulation. Litter derived from the standing vegetation can act as a seed trap and thus prevent seed germination. Non-germinated seeds in the soil are the prime determinant of the composition of the seed bank, and development of a thick layer of litter may promote seed bank development (Egawa et al., 2009).

The Loess Plateau in China is characterized by an arid to semiarid climate. Heavy rain storms easily erode the loess soil, and low vegetation cover and degradation of grasslands by overgrazing have long been a serious problem that has hugely impacted grassland animal husbandry and ecological security in this area (Unkovich and Nan, 2008; Zhao et al., 2013). To prevent further soil erosion and desertification and to protect and restore the grassland, the Chinese government implemented the Returning Farmland to Grassland and Returning Rangeland to Grassland programs in 2000 and 2003, respectively (Lin et al., 2013). However, the effects of restoration programs on grasslands are controversial. Some studies showed that ecological restoration programs had a positive effect on vegetation (Pettit et al., 1995; Liu et al., 2008), and others indicated that reasonable grazing or mowing was conducive to maintenance of the natural vegetation (Dumont et al., 2009; Cheng et al., 2014). Further, long-term enclosure had a negative effect on plant regeneration in semiarid areas on the Loess Plateau due to the accumulation of litter that inhibited seed germination and thus indirectly affected natural regeneration (Cheng et al., 2006). Thus, litter accumulation during ecological restoration may play a key role in the productivity and health of the grassland ecosystem on the Loess Plateau. Understanding the role of litter in regulating seed germination, seedling recruitment and the soil seed bank will aid in developing a new set of indicators for grassland ecosystem health.

Yet, there is little information (Rotundo and Aguiar, 2005; Liu et al., 2016) on how amount of litter and seed position in the litter jointly influence seedling emergence and seed persistence; specifically, no such study has been conducted on the Loess Plateau. *Stipa bungeana*, a dominant perennial grass on the Loess Plateau, plays an important role in protecting the soil from erosion and reducing water loss by runoff (Hu et al., 2014). *Setaria glauca*, a summer annual grass, is native to Eurasia (Steel et al., 1983), but it has become a cosmopolitan grass weed throughout the temperate region (Culpepper and Sheldon, 1999) and is common in the study area. *Lespedeza davurica*, a C₃ perennial leguminous shrub, is a dominant species in the natural grassland community on the Loess Plateau of China (Xu et al., 2013). Also the dominant species *S. bungeana* produces a relatively high amount of litter during the growing season. Thus, we select these three common species and the litter of *S. bungeana* as object to answer the following questions. (1) What amount of litter is beneficial for seedling emergence and seed persistence of the dominant species *Stipa bungeana* and its accompanying species *Setaria glauca* and *Lespedeza davurica*? (2) How do seed position and its

interaction with amount of litter affect seedling emergence and seed persistence?

MATERIALS AND METHODS

Study Site and Species

Field experiments were conducted from 28 July 2013 to 28 December 2014 on Yuzhong Campus (N35°57', E104°09', 1720 m above sea level) of Lanzhou University, Gansu Province, China. Precipitation and temperature data collected on the Yuzhong Campus showed that mean annual precipitation is 350 mm, 60% of which occurs from July to September, and mean annual temperature is 6.7°C.

Seed Collection

Seeds of *S. bungeana* and *S. glauca* were collected at the Yuzhong experimental station in July 2013 and those of *L. davurica* at the same site in October 2012. Seeds of each of the three species were collected from more than 50 individuals and kept in paper bags at room temperature (20–45% RH; 18–25°C) until used in experiments. Other than the after-ripening that may have occurred during storage, no dormancy-breaking treatments were given to the seeds before they were used in the experiments. The 1000-seed mass determined using eight replicates of 100 seed samples was 1.14 ± 0.04 g, 3.56 ± 0.05 g and 1.86 ± 0.04 g for *S. bungeana*, *S. glauca*, and *L. davurica*, respectively.

Seedling Emergence: Effect of Seed Position in Litter, Species and Amount of Litter

The effects of seed position in litter, species and amount of litter on seedling emergence were studied in the field on the Yuzhong Campus from 28 July to 6 October 2013, during the rainy season. Cheng et al. (2006) indicated that the amount of litter was 40–90, 80–140, 120–280, and 160–240 g/m² in *Stipa bungeana*-dominated grassland enclosed for 0–5, 6–10, 11–15, and 16–20 years, respectively, and that it peaked 267 g/m² for this grassland enclosed for 11–15 years. Thus, we used 0, 80, 160, and 240 g/m² to mimic the natural conditions. There were seven treatments: control (no litter, seeds placed on bare soil), seeds on top of 80 g/m² litter layer, seeds beneath the 80 g/m² litter layer, seeds on top of 160 g/m² litter layer, seeds beneath 160 g/m² litter layer, seeds on top of 240 g/m² litter layer and seeds beneath 240 g/m² litter layer. Thus, there were three seed positions (on top of the litter layer, beneath litter layer, on bare soil = no litter) and four amounts of litter (0, 80, 160, 240 g/m²). One hundred and five 20-cm long × 18 cm diameter PVC collars (pots) (about 5.0×10^3 cm³) were used, and each treatment was replicated 15 times (7 treatments × 15 replications = 105 pots). Pots were filled with soil from the natural habitat that had been screened through a 40-mesh screen to remove any seeds of the three study species present in the seed bank. Fifty seeds of each of the three species were sown in 105 pots (150 seeds per pot) on 28 July 2013. Any seeds of *S. bungeana* in the litter

were removed before the litter was used. After seeds and litter were added to the pots, they were covered with 15 cm × 15 cm 40-mesh screen to prevent unintentional seed input and seed predation. The bottom of each pot was covered on the outside with nylon mesh to prevent loss of seeds through the drainage holes. The number of seedlings that emerged was recorded each week for 10 weeks, and seedlings were removed from the pots after they were counted. Seedlings of the two grasses were distinguished based on width of the leaf: leaves of *Stipa* were narrow like needles and those of *Setaria* were significantly wider than those of *Stipa*.

Seed Persistence: Effect of Seed Position in Litter and Amount of Litter

This experiment began on 28 July 2013 and ended 28 December 2014. The experimental design was the same as that described for seedling emergence. Seeds were retrieved from the pots on 28 December 2013, 28 June 2014 and 28 December 2014. For each species, seven treatments with five replicates each, i.e., 35 pots, were retrieved on each of the three dates, and the soil within each pot was sieved through a 40-mesh screen. Then, the number of seeds of each species remaining was counted and tested for viability via germination tests and the embryo cut test. Seeds with white and firm embryos were considered to be viable and those with soft and tan embryos non-viable. Dead non-germinated and dead germinated seeds could not be distinguished from each other.

Statistical Analysis

A three-way ANOVA was used to test the effect of seed position in litter, species and amount of litter on seedling emergence and effect of seed position in the litter, duration in the field (time since sowing) and amount of litter on seed persistence in soil. Percentages of viable seeds were log transformed before analysis, but only non-transformed data are shown in tables and figures. Duncan's multiple range tests were used to compare means among treatments. Data were analyzed using SPSS 21.0 software and the figures were created with Excel 2007.

RESULTS

Seedling Emergence: Effect of Seed Position in Litter, Species and Amount of Litter

Seed position in litter, species, amount of litter and their interactions had significant effects on cumulative seedling emergence. In general, seedling emergence of the three study species differed significantly (Table 1, $P < 0.001$). Seedling emergence of *S. bungeana* and *S. glauca* decreased with an increase in amount of litter for seeds sown on top of litter. However, seedling emergence of *L. davurica* did not differ significantly among different amounts of litter for seeds on top of the litter layer. For seeds sown beneath the litter layer, cumulative seedling emergence of each of the three species was significantly

TABLE 1 | Effect of seed position (P), species (S), amount of litter (L) and their interactions on cumulative seedling emergence.

Source of variation	df	Mean square	F	P
Position (P)	1	10692.900	374.413	<0.001
Species (S)	2	6671.233	233.594	<0.001
Amount of litter (L)	3	578.885	20.270	<0.001
P × S	2	1922.533	67.318	<0.001
P × L	3	1327.152	46.470	<0.001
S × L	6	127.919	4.479	<0.001
P × S × L	6	261.930	9.171	<0.001

higher than that for seeds sown on top of the litter layer or directly on the bare soil (control = no litter) (**Figure 1**).

The highest cumulative seedling emergence of *S. bungeana*, *S. glauca*, and *L. davurica* was $36.13 \pm 3.64\%$, $76.00 \pm 2.25\%$ and $31.47 \pm 3.04\%$, respectively, for seeds sown beneath 160, 240, and 160 g/m² of litter, respectively. For *L. davurica*, seed position in the litter had a significant effect on cumulative seedling emergence, which was lower for seeds on top of the litter layer and on bare ground than for those positioned beneath the litter layer (**Figure 1**).

Seed Persistence: Effect of Seed Position in Litter and Amount of Litter

For all three species, percentage of viable seeds in the soil decreased significantly with an increase of time (duration) in the field (**Figure 2**). Seed position, seed duration in the field (time since sowing), amount of litter and some of their interactions (duration × amount of litter and position × duration × amount of litter) had significant effects on seed persistence of *S. bungeana* (**Table 2**) and *S. glauca* (**Table 3**). However, seed position and seed position × duration × amount of litter did not have a significant effect on seed persistence of *L. davurica* (**Table 4**).

Percentage of viable seeds in the field varied with seed position in litter, seed duration in the field and amount of litter. Percentage of viable seeds of *S. bungeana* was significantly higher for seeds on top of the litter layer than it was for those positioned beneath the litter layer or on bare ground, and it increased with an increase in amount of litter. After 5 months, 37.2% of *S. bungeana* seeds positioned on top of 240 g/m² litter were viable, whereas only 5.2% of those positioned beneath the same amount of litter were viable (**Figure 2**). Percentage of viable seeds of *S. glauca* in the field was significantly affected by seed position. After 5 months in the field, 46.4–53.2% of seeds positioned on top of the litter layer were viable, but only 15.2–28.4% of those positioned beneath different amounts of litter were viable. Decrease in number of viable seeds of *S. glauca* in the field was slower with an increase in time of burial compared to the other two species, and 2.4–12.4% seeds were still viable after 1.5 year under different treatments (**Figure 2**). In contrast to *S. glauca* and *S. bungeana*, seed position of *L. davurica* did not have a significant effect on seed persistence. Percentage of viable seeds of *L. davurica* decreased quickly and was $\leq 3.6\%$ for all the treatments after duration of 11 months in the field (**Figure 2**).

DISCUSSION

Seedling Emergence: Effect of Seed Position in Litter, Species and Amount of Litter

The effect of litter on seed germination and seedling establishment in grasslands ranged from strongly negative to slightly positive depending on environmental conditions, amount of litter and seed traits (Loydi et al., 2013). Our study showed that the effect of litter on seedling emergence ranged from slightly positive (*S. bungeana*) to positive (*S. glauca* and *L. davurica*) for seeds beneath the litter layer compared to those on bare soil. For seeds positioned on top of the litter layer, seedling emergence was slightly promoted, unchanged or inhibited depending on species and amount of litter compared to those on bare ground. Seed position in litter, species (seed traits) and amount of litter had significant effects on seedling emergence of the three common species on the Loess Plateau.

Low to medium amounts of litter (<500 g/m²) had a positive effect on seedling recruitment in dry grasslands or under water-limited conditions (Loydi et al., 2013). This is consistent with our study, which clearly showed that litter had a positive effect on seedling emergence of all three study species when seeds were beneath the litter layer compared to those on bare soil. However, the beneficial effect for seeds beneath the litter layer varied with litter amount and species. For example, seedling emergence increased and then decreased as amount of litter increased in *S. bungeana* and *L. davurica*. However, a continual increase in seedling emergence with an increase in litter amount in *S. glauca* was observed. A possible interpretation of this result is that the presence of litter may maintain soil moisture or reduce the intensity of desiccation of seeds, which would facilitate germination and seedling establishment in a dry environment or during drought (Boeken and Orenstein, 2001; Eckstein and Donath, 2005; Rotundo and Aguiar, 2005; Donath and Eckstein, 2010; Loydi et al., 2013). Further, seedlings from seeds beneath the litter layer must reach light levels sufficient for photosynthesis, and a thick litter layer may reduce seedling emergence due to the lack of light (Facelli and Pickett, 1991b; Wellstein, 2012). Compared with small-seeded species, large-seeded species such as *S. glauca* can better cope with a thick litter layer (Krenova and Leps, 1996; Loydi et al., 2013), since they have sufficient resources for elongation of the hypocotyl to penetrate the litter layer and thus reach full sunlight (Hamrick and Lee, 1987).

However, when seeds were placed on top of the litter layer, seedling emergence was promoted, unchanged or inhibited depending on species and amount of litter. Seedlings from seeds on top of the litter layer must quickly make contact with the soil to order to avoid lethal desiccation (Hamrick and Lee, 1987). Seedling emergence of *S. bungeana* for seeds on top of 80 g/m² litter layer was slightly higher more than that of seeds sown on bare soil, indicating that resource investment for elongation of the radical allows *S. bungeana* to cope with this low amount of litter cover. For *L. davurica*, seedling emergence for seeds on top of the litter layer did not differ significantly

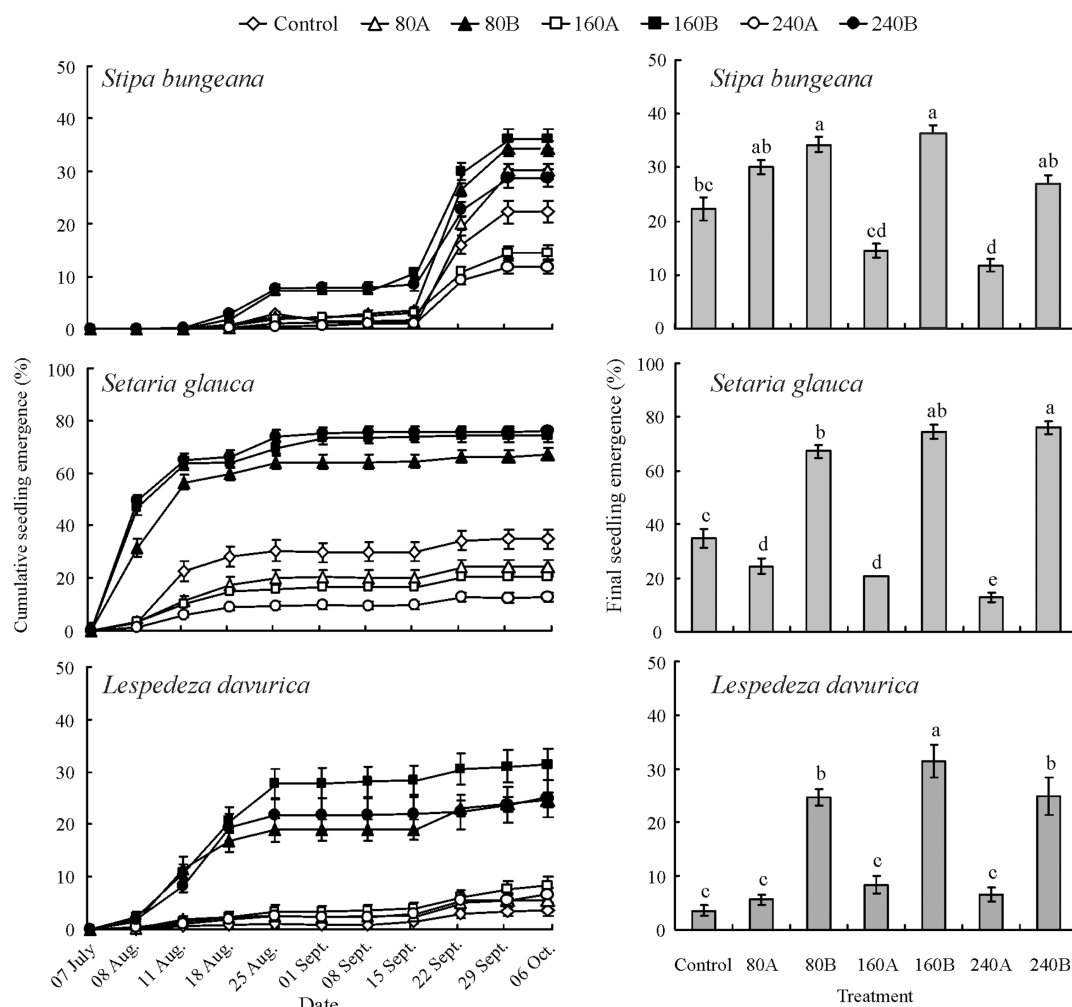


FIGURE 1 | Effect of litter on cumulative seedling emergence (mean% ± SE) of *Stipa bungeana*, *Setaria glauca*, and *Lespedeza davurica*. Bars with different lowercase letters differ significantly among different treatments within the same species. Zero (control), 80, 160, and 240 refer to amount of litter (g/m²), and A and B to seed positioned on top of (A) or beneath (B) the litter layer.

from those on bare soil. This showed that the positive effects of litter, such as attenuating temperature extremes and reducing water stress (Eckstein and Donath, 2005; Donath and Eckstein, 2010), was equal to the negative effect of litter, such as reducing soil-seed contact (Fowler, 1986; Chambers, 2000; Wellstein, 2012). However, the negative effects of litter outweighed its possible positive effects for *S. glauca*, and seedling emergence was significantly inhibited for seeds on top of the litter layer. Our findings highlight that the amount of litter in grassland ecosystems may serve as a potential indicator of plant diversity and composition over time due to its effects on timing of seed dispersal and seedling emergence.

Seed Persistence: Effect of Seed Position in Litter and Amount of Litter

Litter can modify soil temperature and soil moisture (Mackinney, 1929; Fowler, 1986; Boeken and Orenstein, 2001), and previous studies indicated that seed persistence can be affected by soil

temperature, water potential and light transmission through their influence on germination, dormancy and aging (Long et al., 2009, 2015; Pakeman et al., 2012). Litter increased seed longevity of *Bromus pictus* through ameliorating temperature, which is one of the principal drivers of seed aging (Rotundo and Aguiar, 2005).

The presence of a litter layer may maintain soil moisture (Fowler, 1986; Boeken and Orenstein, 2001) and reduce temperature (Chambers and MacMahon, 1994), and temperature and moisture content are important factors that influence seed aging (Walters, 1998). Compared to seed position on top of the litter layer, its position beneath the litter layer had higher moisture content and lower temperature, and the moisture content of a seed at a given relative humidity increases as the temperature decreases (Roberts and Ellis, 1989; Facelli and Pickett, 1991b; Walters, 1998). Lower soil temperature and water availability promote persistence of desiccation-tolerant seeds (Long et al., 2009, 2015; Pakeman et al., 2012) by providing conditions that minimize seed aging, dormancy loss (Davis et al.,

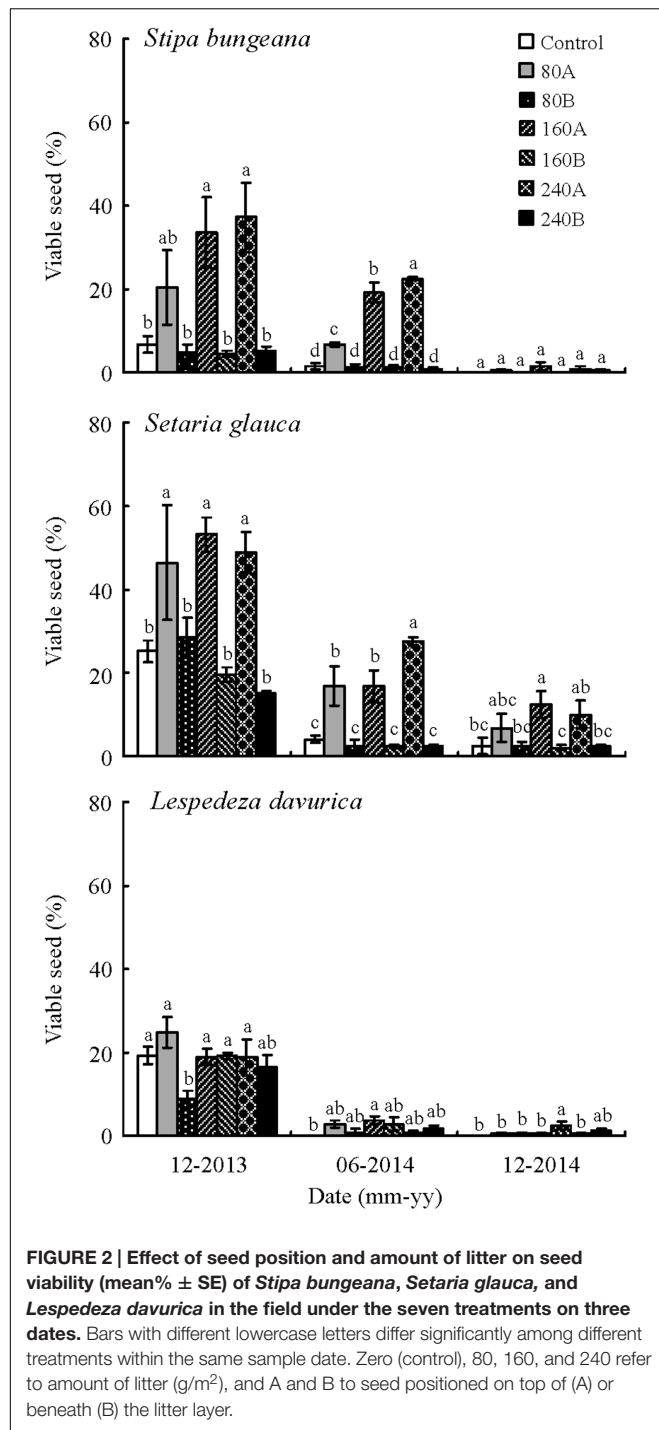


FIGURE 2 | Effect of seed position and amount of litter on seed viability (mean% \pm SE) of *Stipa bungeana*, *Setaria glauca*, and *Lespedeza davurica* in the field under the seven treatments on three dates. Bars with different lowercase letters differ significantly among different treatments within the same sample date. Zero (control), 80, 160, and 240 refer to amount of litter (g/m²), and A and B to seed positioned on top of (A) or beneath (B) the litter layer.

2005) and microbial activity (Schafer and Kotanen, 2003). Thus, the effect of litter on seed persistence depends on whether temperature or soil moisture has a greater effect on seed longevity. Our study showed that on the Loess Plateau seeds of *S. bungeana* and *S. glauca* on top of the litter layer persist longer than those on the bare soil or beneath the litter layer, which indicates that soil moisture plays a more important role in seed persistence than temperature.

TABLE 2 | Three-way ANOVA of the effects of seed position (P), duration (D), amount of litter (L), and their interaction on seed viability of *Stipa bungeana* in the field.

Source of variation	df	Mean square	F	P
Position (P)	1	6.220	83.075	<0.001
Duration (D)	2	7.316	97.712	<0.001
Litter amount (L)	3	0.664	8.870	<0.001
P \times D	2	1.105	14.756	<0.001
P \times L	3	0.847	11.306	<0.001
D \times L	6	0.091	1.218	0.304
P \times D \times L	6	0.158	2.114	0.058

TABLE 3 | Three-way ANOVA of the effects of seed position (P), duration (D), amount of litter (L), and their interaction on seed viability of *Setaria glauca* in the field.

Source of variation	df	Mean square	F	P
Position (P)	1	4.279	42.054	<0.001
Duration (D)	2	8.950	87.963	<0.001
Litter amount (L)	3	0.581	5.708	0.001
P \times D	2	0.647	6.363	0.003
P \times L	3	0.520	5.113	0.003
D \times L	6	0.157	1.542	0.173
P \times D \times L	6	0.100	0.985	0.440

TABLE 4 | Three-way ANOVA of the effects of seed position (P), duration (D), amount of litter (L), and their interaction on seed viability of *Lespedeza davurica* in the field.

Source of variation	df	Mean square	F	P
Position (P)	1	0.010	0.237	0.627
Duration (D)	2	14.817	349.016	<0.001
Litter amount (L)	3	0.255	6.010	0.001
P \times D	2	0.214	5.029	0.008
P \times L	3	0.207	4.867	0.003
D \times L	6	0.117	2.754	0.016
P \times D \times L	6	0.044	1.027	0.413

Percentage of viable seeds of *S. bungeana* was 20.4, 33.6, and 37.2% for seeds positioned on top of 80, 160, and 240 g/m² litter layer, respectively, after 5 months, whereas only 6.8% of those on bare soil were viable. After 11 months, percentage of viable seeds was 6.8, 19.2, and 22.4% for seeds positioned on top of 80, 160, and 240 g/m² litter layer, respectively, all of which were significantly higher than those beneath different amounts of litter layer and on bare soil (1.6%). Hu et al. (2014) reported that all *S. bungeana* seeds had lost viability after 5 months of burial at 5 cm, whereas 12 and 4% of those on the soil surface were viable after 5 and 12 months, respectively. Our results indicated that litter had a positive effect on seed persistence, and percentage of viable seeds increased with amount of litter for seeds on top of litter layer. One possible interpretation of this result is that seeds of *S. bungeana* have a long awn that causes them to be easily trapped in the litter, thus preventing seed contact with the surrounding soil. As such, the seeds failed to germinate or germination was delayed (Ruprecht and Szabo, 2012).

Seeds of *S. glauca* have been reported to live for 15 (Dawson and Bruns, 1975), 30 (Kivilaan and Bandurski, 1981) and 38 (Toole and Brown, 1946) years in soil. Thus, they can form a long-lived persistent seed bank. However, in our study seeds of *S. glauca* lost viability after 11 months. Seedling emergence of *S. glauca* from August to October was >67% for seed beneath the litter layer with different amounts of litter, and it was 12.7% even for those on top of 240 g/m² litter layer. As time increases, most *S. glauca* seeds could germinate if exposed to light and adequate soil moisture. Seeds of this species buried under natural temperatures in Kentucky (USA) in November and exhumed in June and July germinated to 70–100% in light and darkness at 15/6, 20/10, 25/15, 30/15, and 35/20°C (Baskin et al., 1996).

Seed persistence of *S. glauca* and *S. bungeana* was affected by amount of litter and seed position in the litter layer. However, seed persistence of *L. davurica* was affected by amount of litter but not by position in the litter. The large seeds of *S. glauca* and the awned seeds of *S. bungeana* are suspended within the litter or lodged on top of it and thus do not make contact with soil. However, the small seeds of *L. davurica* on top of the litter layer may ‘percolate’ through the litter layer and then germinate when conditions are favorable for them to do so (Rotundo and Aguiar, 2005; Ruprecht and Szabo, 2012). The fact that the soil seed bank drives multiple ecosystem functions related to community dynamics and composition suggests that seed persistence should be taken into account in assessing the impact of changes in land use and climate.

PRACTICAL IMPLICATIONS

The present study clearly demonstrates that litter plays a key role in regulating seed germination, seedling emergence and seed persistence. Specifically, a moderate litter amount

favors seedling emergence, whereas continual increasing litter decrease seedling emergence. These results imply that moderate utilization will be beneficial for vegetation restoration of long-term enclosure grassland, in which the amount of litter is high. Also, we propose that the amount of litter could be a good indicator for effective restoration and for grassland management. Our study showed that maintaining the amount of litter between 80–160 g/m² is optimal for *S. bungeana*-dominated grassland on the Loess Plateau. Moreover, we also found that seeds beneath litter significantly improved seedling emergence regardless of litter amount, suggesting that moderate disturbance favoring downward movement of seeds may accelerate vegetation restoration. Our study supports the notion that litter accumulation resulting from long term enclosure decreases the capability of grassland regeneration and suggests that moderate utilization is necessary for maintaining a healthy grassland ecosystem on the Loess Plateau, with litter as a potential indicator.

AUTHOR CONTRIBUTIONS

RZ, XH, and YW conceived the topic. RZ performed the experiments. RZ and XH analyzed all statistical data. RZ and XH wrote the manuscript. JB and CB revised several version of the manuscript.

ACKNOWLEDGMENT

This work was supported by the Programme of Introducing Talents of Discipline to Universities (B12002), Fundamental Research Funds for the Central Universities (lzujbky-2016-11) and the National Natural Science Fund (31672473).

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Conflict of Interest Statement: 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.

The reviewer NK and handling Editor declared their shared affiliation, and the handling Editor states that the process nevertheless met the standards of a fair and objective review.

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Assessing Ecosystem Services and Multifunctionality for Vineyard Systems

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OPEN ACCESS

Edited by:

Evangelia G. Drakou,
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 14 October 2016

Accepted: 03 April 2017

Published: 21 April 2017

Citation:

Winkler KJ, Viers JH and Nicholas KA
(2017) Assessing Ecosystem Services
and Multifunctionality for Vineyard
Systems. *Front. Environ. Sci.* 5:15.
doi: 10.3389/fenvs.2017.00015

Vineyards shape important economic, cultural, and ecological systems in many temperate biomes. Like other agricultural systems, they can be multifunctional landscapes that not only produce grapes, but also for example serve as wildlife habitat, sequester carbon, and are places of rich traditions. However, research and management practices often focus mostly on individual, specific ecosystem services, without considering multifunctionality. Therefore, we set out to meet four research objectives: (1) evaluate how frequently the ecosystem services approach has been applied in vineyard systems; (2) identify which individual ecosystem services have been most frequently studied in vineyard systems, (3) summarize knowledge on the key ecosystem services identified in (2), and (4) illustrate approaches to multifunctionality in vineyards to inform more holistic land management. For research objective (1), we identified 45 publications that used the term “ecosystem services” in relation to vineyards, but found that only seven fully apply the ecosystem service concept to their research. For research objective (2), we operationalized the Common International Classification of Ecosystem Services (CICES) for 27 ecosystem services in vineyards, in order to consider provisioning, regulating, and cultural services through an analysis of more than 4,000 scientific papers that mentioned individual services. We found the six most frequently studied ecosystem services included (1) cultivated crops, (2) filtration, sequestration, storage and accumulation by the vineyards, (3) pest control and (4) disease control, (5) heritage, cultural and (6) scientific services. For research objective (3), we found that research on these six single ecosystem services is highly developed, but relationships between single ecosystem services are less studied. Therefore, we suggest that greater adoption of the ecosystem services approach could help scientists and practitioners to acknowledge the multifunctionality of the agricultural system and gain a holistic perspective that supports more sustainable land management.

Keywords: vinecology, multifunctional agriculture, ecosystem services bundles, operationalization, wine, viticulture

INTRODUCTION

Agricultural landscapes provide a multitude of services and benefits to people and nature (Lovell and Taylor, 2013; Galler et al., 2015), including their critical roles in food production, but also to enhance rural livelihoods and ensure functional ecosystems (Hart et al., 2016). Thus, many agricultural systems are examples of multifunctional landscapes, which provide multiple ecosystem services (Lovell et al., 2010; Bennett, 2017). Multifunctional landscapes are often more resilient to ecosystem shocks and disturbances, such as human-induced changes like deforestation, or climate-induced environmental variation (DeClerck et al., 2016). They provide a wider range of services, which can be beneficial when reacting to future challenges, such as climate change or water scarcity, as they can either buffer disturbances or offer alternatives to current situations (Lovell and Taylor, 2013). There is increasing recognition that land management balancing a wide variety of functions is critical to meet the Sustainable Development Goals, especially as they may present trade-offs and interlinkages (Nilsson et al., 2016).

Despite the benefits of recognizing and pursuing land management strategies that consider the multifunctionality of the landscape to meet multiple desired human and ecosystem goals, this is rarely done in practice. So far, the most common approach is to concentrate on one service (e.g., high crop yields from high-intensity farming) and ignore the potential for other ecosystem services in the landscape, such as regulating and cultural services. For example, many ecosystem service studies identify, quantify, and evaluate a single identified service, such as carbon sequestration, without considering the impact on related services (Seppelt et al., 2011; Lee and Lautenbach, 2016). This single-minded focus, either in scientific inquiry or management activity, neglects the importance of the range of services and benefits the land can provide if managed holistically.

The ecosystem services approach pursues an holistic view on ecosystems and their benefits to people (Everard, 2015), which can help to achieve multifunctional landscapes through recognizing and managing a comprehensive range of ecosystem services. Land managers often aim to optimize across competing endpoints, such as maximizing crop quality or production and minimizing labor or inputs, thereby creating trade-offs among different ecosystem services and management priorities. This can overshadow potential benefits of having multiple ecosystem services if such services are in competition, neglected, or unrecognized. For example, in the Napa River winegrowing region of California, USA, maximizing vineyard production area resulted in the reduction of riparian vegetation, which in turn reduced the positive benefits of pest and disease control agents hosted in such habitats (Baumgartner et al., 2006).

While it is likely not possible to achieve all desired ecosystem management outcomes at a maximal level (*sensu* Foley et al., 2005), it may be possible to achieve multifunctional agricultural landscapes providing a plentitude of ecosystem services. To date, however, the range of ecosystem services available in such landscapes is poorly understood, limiting the potential to manage landscapes to optimize their benefits.

Vineyards are important economic, cultural, and ecological systems in many temperate biomes (Figure 1). Globally, the 7.5 million hectares of vines produce about 75.7 million tons of grapes annually, which are used for wine (ca. 45%), as table grapes (ca. 36%) and dried grapes (ca. 8%) OIV, 2016. In 2016, 258 million hectoliters of wine were produced worldwide and the total value of exported wines was €28 billion (OIV, 2016). In some areas in high producing wine countries such as Spain, Italy and France, more than 20% of the agricultural land is under vines (EC, 2009). As perennial agricultural systems, vineyards shape the appearance of whole landscapes, create unique ecosystems as well as cultural traditions (Daniel et al., 2012). The UNESCO has conferred the title of World Heritage Site to multiple winegrowing areas, such as the Piedmont in Italy and Burgundy in France.

In order to examine an agricultural system that provides documented provisioning, regulating, and cultural ecosystem services, we set out to achieve four objectives: (1) evaluate how frequently the ecosystem services approach has been applied in vineyard systems; (2) identify which individual ecosystem services have been most frequently studied in vineyard systems; (3) summarize knowledge on the key ecosystem services identified in (2); and (4) illustrate approaches to multifunctionality in vineyards.

We approach the first two research objectives through operationalizing the Common International Classification of Ecosystem Services (CICES; Haines-Young and Potschin, 2012) framework for vineyards to guide a systematic review of academic literature. To our knowledge, CICES has not previously been applied in a comprehensive and consistent way to design a literature analysis of an agricultural landscape. Using the insights gained as a starting point, we then synthesize how the identified six key ecosystem services have been studied to date. Last, we elaborate how the six key ecosystem services relate to each other to inform more holistic land management. Our overall goal was to understand how the ecosystem services concept is currently applied in studying vineyard landscapes, and examine the potential for promoting a multifunctional perspective in the future.

METHODS

In order to achieve the first and second objectives, we conducted a structured literature search in the Scopus database for peer-reviewed literature in English including all available publications until July 2016. Scopus covers publications back to 1823, but more than 60% of the records are post-1995 (Elsevier, 2016). For the first objective, we looked for publications that specifically used the term “ecosystem services” or synonyms in combination with a variety of possible search terms connected to winegrowing (e.g., “viticulture,” “vineyard”) in their title, keywords, or abstract (Figure 2).

However, researchers frequently study one or more ecosystem services without specifically using the term “ecosystem services.” A study may for example examine the effect of integrated pest management (regulating service), without referring to

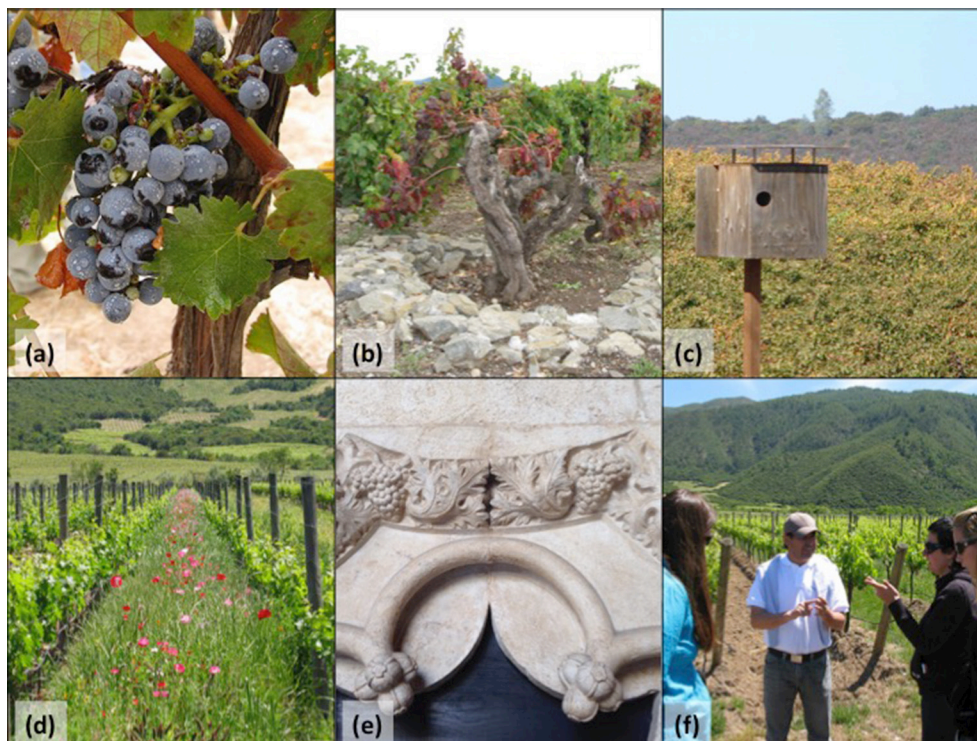


FIGURE 1 | Pictures illustrating the six key ecosystem services we identified in vineyards. Top row (left to right): **(a)** winegrapes (*cultivated crops*); **(b)** vines storing carbon (*sequestration*); **(c)** owl box for rodent control (*pest control*). Bottom row **(d)** providing habitat for beneficial insects (*disease control*); **(e)** grapes motif decorating 500 years old monastery in Portugal (*heritage*); **(f)** vineyards as research grounds (*scientific*). [pictures: KAN **(a)**; JHV **(b,c,d,f)**; KJW **(e)**].

“ecosystem services.” For our second research objective, we selected 27 of the 42 CICES ecosystem services classes most relevant for vineyards to capture such studies (**Figure 2**). We excluded the other 15 ecosystem services classes in the CICES classification (for example, *surface water for drinking*) because we deemed them not relevant for vineyards.

In order to operationalize the ecosystem services classes, we identified search terms for each ecosystem services class based on our expertise in research on vineyards as an author team (e.g., Nicholas et al., 2011; Viers et al., 2013; Winkler and Nicholas, 2016). For example, we started with the CICES ecosystem services class *cultivated crops* under the provisioning section, and developed a list of 15 search terms that were specific to grape and vineyard crop cultivation, continuing through each of the 27 classes (**Table 1**). We then used these search terms to conduct the literature search in Scopus to identify papers that had these search terms appearing in the title, keywords, or abstract; we did not analyze the full text of each publication to ascertain that the publications really did deal with the specific ecosystem service in vineyards. We assumed that search terms returning more than 2,000 hits were too general to be useful and were therefore refined to be more specific, with the original results excluded from further analysis.

Our second objective was to identify the most frequently studied ecosystem services using these keyword searches. Therefore, we wanted a robust measure for the frequency that

each service appeared in the scientific literature. We assumed that if the same publication was found twice or more often for the same ecosystem service using different search terms, it was more likely that the publication really dealt with the specific ecosystem service. Because we were able to identify more search terms for some services than others, and we recognized that the number of search terms used could bias how many publications were found for each service, we calculated ratios. We compared the “hits” (the number of publications returned for each search for the different ecosystem services in vineyards) in four ways: (1) the number of hits, (2) a ratio of the number of hits and the number of search terms, (3) the number of papers that were found at least twice for one ecosystem service by using different search terms within one service class (multiple hits), and (4) a ratio between the multiple hits and the number of search terms. In the end, we judged the most researched ecosystem services as those ranked high in three of our four ranking systems.

We approached our third research objective by using the literature captured from the first two research objectives to briefly describe the six key ecosystem services in vineyard systems, to capture what is already known about these services and identify potential research gaps. We also created a visual snapshot (word cloud) of the key terms associated with each of the key ecosystem service in vineyards we identified in the second research objective, based on the abstracts of all of the papers identified for each ecosystem service.

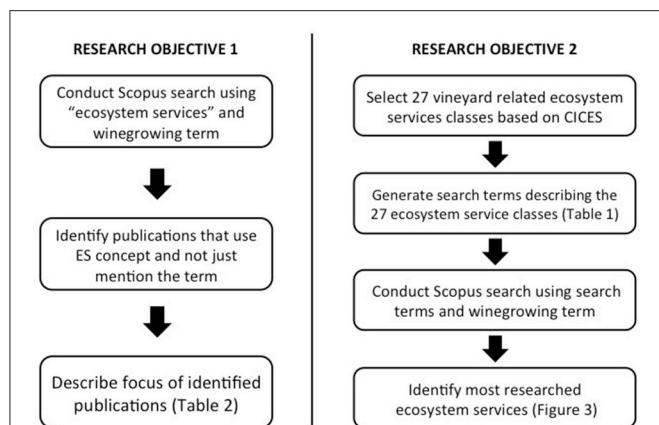


FIGURE 2 | Overview of the research process for the first and second research objective. The first search looked for publications focused on applying the ecosystem services concept in vineyard landscapes by searching for a combination of the term “ecosystem services” and winegrowing terms (e.g., “vineyard,” “viticulture,” “wine,” “grape” grow*, “wine” grow*), identifying seven papers that specifically focused on vineyard ecosystem services, which were described in **Table 2**. The second search looked for publications researching single ecosystem services by operationalizing 27 CICES ecosystem services classes for vineyards (shown in **Table 1**) and generating search terms for each ecosystem service in order to conduct a literature search for peer-reviewed publications to identify the most researched ecosystem services (shown in **Figure 3**).

Last, we selected different ways to research the fourth research objective, where we wanted to illustrate multifunctionality in vineyards. First, we looked for papers that appeared in two or more ecosystem service classes. Since each service had a different number of publications identified (**Table 1**), we calculated the number of papers overlapping relative to each service. In addition, we draw on the literature and previous knowledge to elaborate on relationships between the six key ecosystem services, and also the different scales of the vineyard system they appear in.

RESULTS

Objective 1: Papers Applying the Ecosystem Services Approach in Vineyards

From the literature search on the term “ecosystem services” and vineyards, we found 45 publications specifically addressing ecosystem services and vineyards. We read all of these papers in full and judged that only seven fully applied the ecosystem services concept to their research (**Table 2**). For example, Fiedler et al. (2008) operationalize the concept studying how habitat management affects ecosystem services such as *pest control*, *soil quality*, *water quality*, and *aesthetics* in vineyards. Six of these seven papers focused on regulating and maintenance ecosystem services, particularly *pest control*, but also *carbon sequestration* and *soil characteristics*. Two articles dealt with cultural ecosystem services (Fiedler et al., 2008; Winkler and Nicholas, 2016) and only one directly studied grape yields termed as a provisioning ecosystem service (Kross et al., 2012). However, most of these papers discussed regulating and maintenance ecosystem services

as important to allow high crop yields, thereby drawing a connection to provisioning ecosystem services.

The remaining 38 publications that mentioned the term ecosystem services in their title, keywords or abstract did not further operationalize the concept for their research. For example, Kelly et al. (2016) use “ecosystem services” as a keyword, but do not use or apply it in the text, which instead focuses on bat activity in vineyards.

Objective 2: Identifying Most Studied Vineyard Ecosystem Services

Using the search terms in **Table 1**, the literature search on single ecosystem services resulted in more than 32,000 hits, with the most commonly researched ecosystem services being *cultivated crops* and *disease control*, both with around 4,000 raw hits (**Figure 3**; **Table 1**). The regulating ecosystem services *filtration/sequestration/storage/accumulation by ecosystems* (hereafter called “*sequestration*”) and *pest control*, and the cultural service *scientific*, each had between 2,500 and 3,000 raw hits (**Table 1**). To factor in the varying numbers of search terms, we calculated the ratio between the raw hits and the number of search terms: the ecosystem service classes *scientific*, *disease control*, and *cultivated crops* had high ratios, meaning that regardless of the amount of search terms used, many publications were identified for these classes. When checking for multiple hits (papers found at least twice for one ecosystem service using different search terms), the three service classes *pest control*, *disease control*, and *cultivated crops* performed well, with more than 1,000 multiple hits (**Figure 3**). For the other calculated ratio (multiple hits per search term), the ecosystem services *disease control* and *cultivated crops* and this time *pest control* resulted in high ratios.

There were few studies for most cultural ecosystem services (entertainment, experiential use, symbolic, aesthetic, bequest, sacred and/or religious, and physical use), as well as the provisioning service materials from plants for agricultural use and the regulating service flood protection, with each class containing fewer than 410 hits (**Figure 3**).

In the end, we selected six key ecosystem services as the most widely studied in vineyard systems (**Figure 1**), based on their high ranking in three of our four ranking systems (**Figure 3**): *cultivated crops*, *sequestration*, *pest control*, *disease control*, *scientific*, and *heritage*, *cultural* (hereafter called “*heritage*”). Given that we performed a review of scientific literature, it is not surprising that we identified *scientific* as one of the key ecosystem services commonly studied; this reflects the nature of our approach and provides further evidence that vineyards provide compelling agroecosystems for scientific investigation, especially around the search term climate change.

Objective 3: Knowledge on Key Ecosystem Services in Vineyards

Provisioning Services

Cultivated crops

Cultivated crops include items for direct human consumption like cereals, vegetables and fruits. In connection with vineyard

TABLE 1 | Search terms used to find peer-reviewed papers in Scopus on the three sections of ecosystem services in vineyards and 27 selected ecosystem services classes within them, following the CICES classification (CICES, 2013).

Section	Class	Search terms used to identify papers in Scopus	Search terms	Raw hits	Multiple hits
Provisioning	<i>Cultivated crops</i>	Yield*, grape leaves, grapevine leaves, crop*, table, grape*, crop load*, grape berr*, berry growth, grape maturity, yield component*, fruit composition, cultivated crops*, wine grape*	15	4,024	1,036
	Fibers and other materials from plants, algae and animals for direct use or processing	Pruning, grape seed*, grape skin*, MegaPurple, color additive*, wood, Ravaz index	7	1,209	76
	Materials from plants, algae and animals for agricultural use	Pomace	1	149	149
Regulation and Maintenance	<i>Filtration/ sequestration/ storage/ accumulation by ecosystems</i>	Carbon storage, carbon sequestration, filtration, sequestration, storage, accumulation, GHG, greenhouse gas, N2O, nitrous oxide, sulfur, nitrogen deposition*, fertilizer*, spray, pesticide*, salinization, soil salinity, salt accumulation	18	2,724	353
	Mediation of smell/ noise/ visual impacts	Zoning, spatial planning, smell impact, noise impact, visual impact, smell, planning, land use planning, highway, tractor noise, sulfur smell, harvest, crush smell, landscape, viewshed, preservation, sound cannon*, reflectors	21	1,502	95
	Mass stabilization and control of erosion rates	Soil conservation, soil loss*, cultivation practice*, mass stabilization, erosion, erosion rate, erosion model, alternate row cultivation, row cultivation, disking, mowing, ripping, liming, tree removal, run off, erosivity, land terrac*, native vegetation removal, vegetation removal, cover crop, mass flow, tractor*, machinery	23	544	173
	Hydrological cycle and water flow maintenance	Fraction of Transpirable Soil Water, FTSW, infiltration, water deficit, water relations, hydraulics, run off, soil moisture, irrigation, fish AND flows, ecolog* flow*, water security, water stress	13	925	281
	Flood protection	Flooding, landscape, buffer zone, setback, flood control, flood protection, wet feet, drainage	8	339	10
	Ventilation and transpiration	Evapotranspiration, ventilation, transpiration, photosynthesis, ecophysiology	6	453	84
	Pollination and seed dispersal	Insect*, pollination, seed dispersal, bee, bird*, starling*, arthropod, finch*, cover crop, wind pollination, turkey*, sound cannons*	12	899	78
	Maintaining nursery populations and habitats	Diversity, biodiversity, nursery population, habitat, germplasm, biological resource, gene pool	7	1,008	177
	<i>Pest control</i>	Cover crop, pest*, pest control*, rodent control*, beneficial predator*, bird box*, owl box*, raptor box*, nest box*, integrated pest management*, IPM, native plant*, natural enemy, pest management, pesticide, biological control, arthropod, rodent*, insecticide*, phyloxera, nematode*	21	2,630	1,339
	<i>Disease control</i>	Red blotch, botrytis, fungal, herbicide, phomopsis, disease*, fungicide*, disorder*, eutypa, biological control, fanleaf, mulch, leafroll, corky bark	14	3,984	1,048
	Weathering processes	Soil fertility, nutrient*, soil structure, in situ soil, soil biological activity, nutrient uptake, mineral*, soil quality, weathering process*	9	859	129
	Decomposition and fixing processes	Microbe*, fungi, soil arthropod*, arthropod, mulch, worm*, legume*, nitrogen fixing, soil quality, decomposition, fixing process*	11	1,204	32
	Micro and regional climate regulation	Latent heat, transpiration, climat* regulation, shade, hydrologic cycle, micro climate, regional climate	9	1,695	58
Cultural	Experiential use of plants, animals and land-/ seascapes in different environmental settings	Wine tasting, picnic*, eating grape leaves, drink* wine, dolmade*, birding, bird watch*, employment, hot air, balloon ride, limousin* tour*, gourmet tourism, cable car	12	134	0
	Physical use of land-/ seascapes in different environmental settings	Biking, hiking, horseback rid*, padding, walking	5	8	0
	<i>Scientific</i>	Climate change, enology, trial, precision viticulture, scientific	5	2,947	300
	Educational	Winemaking, winegrowing, wine seminar, school, university, college, education, tasting room, environmental education	9	1,358	68
	<i>Heritage, cultural</i>	Family winery, tradition, charm, traditional, historical, identity, sense of place, social capital, heritage, local food cultural	11	1,485	205

(Continued)

TABLE 1 | Continued

Section	Class	Search terms used to identify papers in Scopus	Search terms	Raw hits	Multiple hits
	Entertainment	Wedding*, entertainment, bachel* part*, winery tour, wine tasting, concert, theater, music, movie*, film festival, festival, harvest festival, contest, vintage festival, wine, queen, wine event*, tourism, agritourism, agrotourism, wine cave, wine tourism, wine tour*, visit, day trip	24	390	172
	Aesthetic	Beauty, scenery, landscape, winescape, vineyard row, aesthetic, mustard, poppies, inspiration, wildflower, seasonal change, leaf change, foliage change, art, gallery	17	393	14
	Symbolic	Representation, appellation, symbolic, social cohesion, terroir, uniqueness, AVA, American Viticultural Area, DOC, denomination origine controllee, denominazione di origine controllata, AOC, Appellation d'origine contrôlée, emblem*	13	406	43
	Sacred and/ or religious	Wedding, yoga, meditation, retreat, spiritual, sacred, religious, religion, mother earth, inspiration	13	77	12
	Existence	View, land use, option value, existence, nature conservation, landscape	6	957	104
	Bequest	Family farming, family winery, inter-generational, stewardship, land ethic, bequest	7	15	0

Individual search terms that resulted in more than 2,000 hits were rephrased. These search terms are crossed in the table. The table shows the number of search terms, the number of raw hits revealed and the number of papers with multiple hits (papers found at least twice between different searches). The six key ecosystem services are shown in red and italics. An asterix (*) indicates a wildcard for searching that would find all variations on that term (e.g., *yield** would include *yield*, *yields*, *yielding*). The six key ecosystem services we identified from the literature review are shown in italics.

systems, they mainly include table and wine grapes harvested from vineyards, as well as vine leaves used for eating in some Mediterranean cuisines (**Figure 4**). Wine growers aim to increase or stabilize both the quality and quantity of the grapes (Kross et al., 2012). While a high yield is one goal, quality aspects such as fruit composition and taste are also important for most grape growers, as quality can contribute to a variation in price of over 10-fold for the same grape variety grown within 320 km (Nicholas, 2015). Nevertheless, the ecosystem service cultivated crops is easy to measure, and various vine and wine associations from the global to regional level publish annual harvest numbers (e.g., OIV, 2016, 2014; UKVA, 2012; Wine Institute, 2013).

Regulating and Maintenance Services

Filtration/Sequestration/Storage/Accumulation by Ecosystems

This ecosystem service describes bio-physicochemical *filtration*, *sequestration*, *storage*, and *accumulation* processes that help to fix pollutants and organic compounds in the soil as a result of a combination of biotic and abiotic factors (CICES, 2013). Like all long-lived perennial systems, vineyards play a role as potential places to sequester carbon, with vineyard research in this category focusing especially on soil (Morandé et al., 2017; **Figure 4**). Naturally, with their lower biomass, vineyards store less carbon than woody wildlands (Kroodsma and Field, 2006). However, the management of vineyards can increase the amount carbon stored in soil and as perennial wood up to 90% (Galati et al., 2016). At the landscape scale, a mixed land cover in vineyards with vines and native natural vegetation increases carbon stocks in the soil, compared with monocultural vineyard management (Steenwerth and Belina, 2008; Williams et al., 2011).

Emissions of nitrous oxide (N₂O), a greenhouse gas 298 times more potent than carbon dioxide (Forster et al., 2007), are found in agricultural systems including many vineyards due to mineral nitrogen fertilizer applications. There are strategies that can be employed to reduce the level of N₂O emissions in vineyards. The most commonsense strategy to mitigate N₂O emissions is to adopt a sustainable nitrogen fertility program. Practices can include spatiotemporal accounting of nitrogen, specific N₂O limitation through timely manure management, use of N-fixing legumes as cover crops in place of synthetic fertilizers, and management of soil carbon and alkalinity to limit undesirable biochemical reactions (Dalal et al., 2003). Further, it has been shown that increased soil C and microbial biomass can elevate N retention in soils (Steenwerth and Belina, 2010), suggesting synergistic benefits from holistic soil management.

Pest Control

Pest control describes natural processes provided by ecosystems that help to reduce and limit pests in the ecosystem. As for all agricultural landscapes, *pest control* is important in vineyards to protect the vines from damage caused by animals attacking the growing vines (e.g., nematodes feeding on grapevine roots, or grapeleaf skeletonizers attacking the leaves), or eating or damaging the crop (e.g., passerine bird damage in New Zealand is a serious economic problem, where up to 83% of a vineyard's crop have been damaged (Kross et al., 2012). Considerable effort and financial resources are dedicated to *pest control* in vineyards, often using pesticides (as appearing in **Figure 4**), especially since pests can easily spread in monocultures.

However, there is mounting evidence that vineyards can benefit significantly from the *pest control* services provided

TABLE 2 | Overview of seven publications fully applying the ecosystem services concept in vineyards, identified through systematic literature search.

Authors	Title	Year	Summarized abstract	Provisioning	Regulating & maintenance	Cultural
Galati et al.	Actual provision as an alternative criterion to improve the efficiency of payments for ecosystem services for C sequestration in semi-arid vineyards	2016	<ul style="list-style-type: none"> Evaluate the efficiency of a PES scheme based on an egalitarian criterion in relation to soil carbon (C) sequestration Result: adoption of an egalitarian criterion generates an inequitable distribution of agri-environmental payments but leads to up to 90% increase soil C sequestration 		Filtration/ sequestration/ storage/ accumulation by ecosystem	All eleven cultural ecosystem services in CICES
Winkler and Nicholas	More than wine: Cultural ecosystem services in vineyard landscapes in England and California	2016	<ul style="list-style-type: none"> Assess perspectives of wine producers and residents regarding cultural ecosystem services provided by vineyards Result: identified eight perspectives on cultural ecosystem services, depending on experience (resident or wine producer) and location (new or established vineyard landscape) 			
Shields et al.	Potential ecosystem service delivery by endemic plants in New Zealand vineyards: Successes and prospects	2016	<ul style="list-style-type: none"> Low-growing, endemic plant species were evaluated for their potential benefits (providing habitat for beneficial species) & disservices (providing habitat for pests) as cover crops Result: enhancing plant diversity in vineyards can harbor ecosystem services providers (e.g. G. G. sessiliflorum) and therefore deliver ecosystem services including pest control 		Pest control Weathering processes	
Kross et al.	Effects of introducing Threatened Falcons into Vineyards on Abundance of Passeriformes and Bird Damage to Grapes	2012	<ul style="list-style-type: none"> Introduction to vineyards of the New Zealand Falcon to control Passeriformes, which are considered vineyard pests that cause economic loss due to grape damage Result: introduction of falcons to vineyards was associated with decrease in abundance of passerines and with a 95% reduction in the number of grapes damaged relative to vineyards without falcons 	Cultivated crops	Pest control	
Jedlicka et al.	Avian conservation practices strengthen ecosystem services in California vineyards	2011	<ul style="list-style-type: none"> Insectivorous Western Bluebirds occupy vineyard nest boxes established by California winegrape growers Result: presence of Western Bluebirds in vineyard nest boxes increases removal rates of harmful insects (e.g., beet armyworms) by up to 3.5 times 		Pest control	
Danne et al.	Effects of native grass cover crops on beneficial and pest invertebrates in Australian vineyards	2010	<ul style="list-style-type: none"> Indigenous cover crops potentially promote increase in natural enemies providing control of pest species Result: native plants have potential to increase beneficial invertebrates assisting pest control, but can also increase local pest problems 		Pest control	

(Continued)

TABLE 2 | Continued

Authors	Title	Year	Summarized abstract	Provisioning	Regulating & maintenance	Cultural
Fiedler et al.	Maximizing ecosystem services from conservation biological control: The role of habitat management	2008	<ul style="list-style-type: none"> • Synergies among biodiversity conservation, ecological restoration, human cultural values, tourism and biological control have largely been overlooked in past habitat management research • Native plants are useful in restoration of rare ecosystems, and can increase natural enemy abundance as much as widely recommended non-natives • Additional ecosystem services provided by habitat management in New Zealand vineyards • Result: multiple ecosystem service goals can decrease agriculture's dependence on 'substitution' methods such as agro-chemical inputs 		Maintaining nursery populations and habitats Pest control Disease control	Physical use of landscape Heritage

by natural enemies (e.g., parasitoids, predators, antagonists, pathogens) of grapevine pests living in the vineyard landscape (Kross et al., 2012). Many vineyards provide good habitat for pests, but provide only minimal food or shelter for natural enemies.

Vineyard management using knowledge of conservation biology and integrated pest management (IPM) can contribute to the reduced need for chemical pesticides (Campos and Zhang, 2004), as well as herbicides used against weeds that compete with the vines. For example, creating habitat to meet the needs of certain animals and plants can strengthen food webs and native biodiversity (Fiedler et al., 2008; Tompkins, 2010; Jedlicka et al., 2011; Orre-Gordon et al., 2013). Integrated pest management aims to reduce the usage of synthetic chemical inputs using existing knowledge of the grapes and possible pests, while enhancing ecosystem services including the *cultivated crops* of the vineyards. This method works by promoting conditions for natural *pest control*; for example, mulching supports beneficial organisms like arthropods (Addison et al., 2013). However, IPM must be practiced carefully, as including native plants can also intensify pest problems (Danne et al., 2010).

Disease Control

The ecosystem service of *disease control* describes the natural reduction or limitation of diseases caused by pathogens. Grapevines are subject to infection from a variety of diseases caused by viral, bacterial, or fungal infections. Disease control is often closely related to *pest control*, as vineyard pests spread many diseases. For example, the glassy-winged sharpshooter is a vector for the deadly bacterium *Xylella fastidiosa* responsible for Pierce's Disease, and the dagger nematode spreads the grapevine fanleaf virus.

Careful vineyard management can provide the service of *disease control* (Figure 4). For example, maintaining natural habitat or a diversity of agricultural crops near vineyards can help provide from *disease control* services in vineyard systems (Shields et al., 2016). Management practices to increase the biological degradation of vine debris can decrease harmful fungus abundance (Jacometti et al., 2007). Pruning, leafing, and other grapevine canopy management strategies improve air circulation and light penetration, which is beneficial for *disease control*. Some growers believe that their efforts to increase the soil quality and improve vine health strengthen the plant's ability to withstand disease pressure, for example phylloxera (Nicholas and Durham, 2012).

Fungal diseases, such as downy mildew, are often correlated with warm and damp weather conditions that favor the growth of rots and molds. Controlling such diseases involves careful weather monitoring to only spray control material when it is most necessary and effective. The powdery mildew Risk Assessment Index program in California, where growers can look up the disease risk online in real time based on local weather conditions and plan their fungicide spraying schedule accordingly, has successfully reduced spraying fungicides by 2–3 times per year, with equally effective disease control (Gubler et al., 1999).

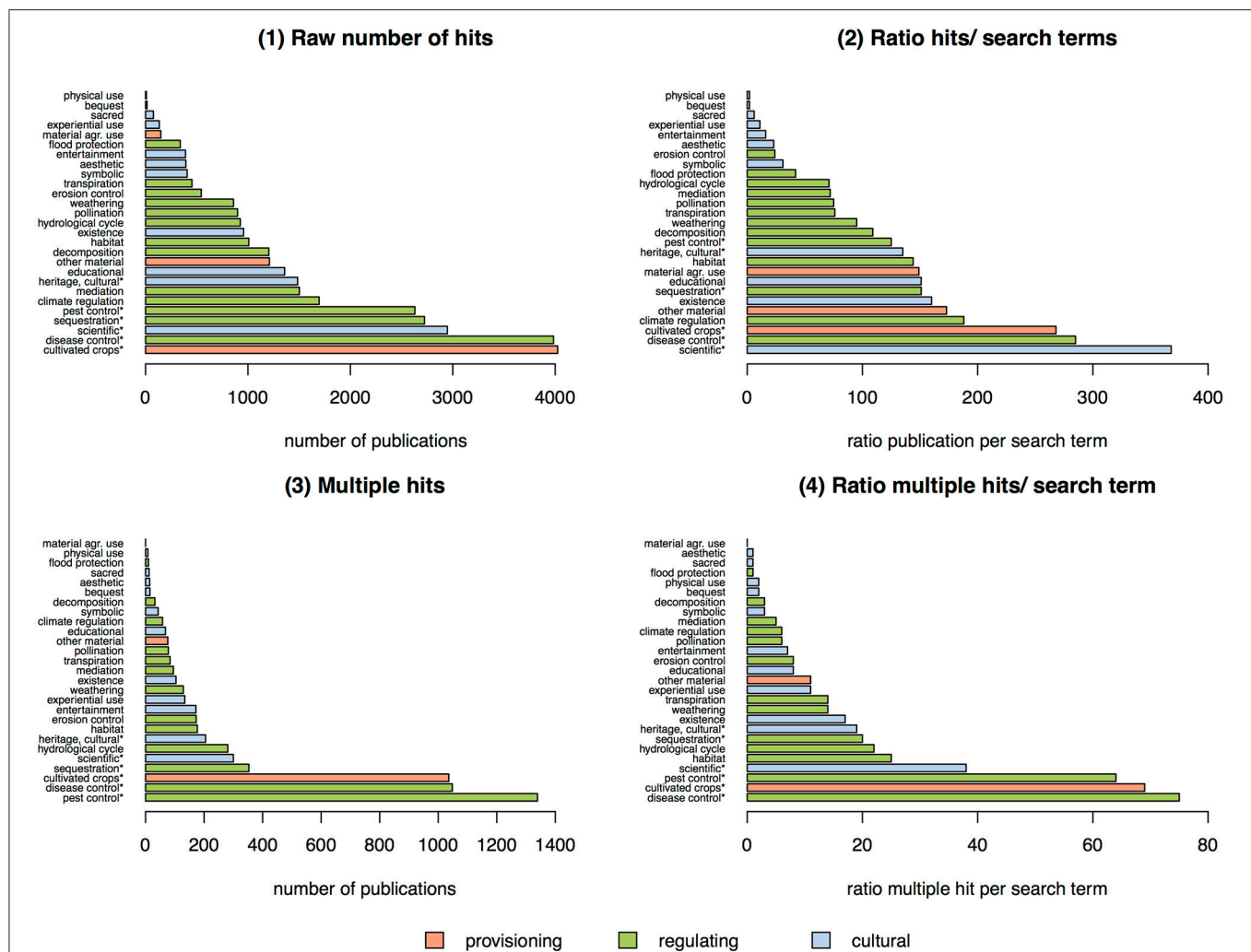


FIGURE 3 | Comparison of publications found for single ecosystem services in vineyards (research objective 2), identified from the full literature review using search terms from Table 1. We compared the “hits” (the number of publications returned for each search for the different ecosystem services in vineyards) in four ways: (1) the raw number of hits, (2) a ratio of the number of hits and the number of search terms, (3) the number of papers that were found at least twice for one ecosystem service by using different search terms within one service class (multiple hits), and (4) a ratio between the multiple hits and the number of search terms. We identified six key ecosystem services as the most widely researched, based on their high rating in at least three out of the four methods (indicated with *).

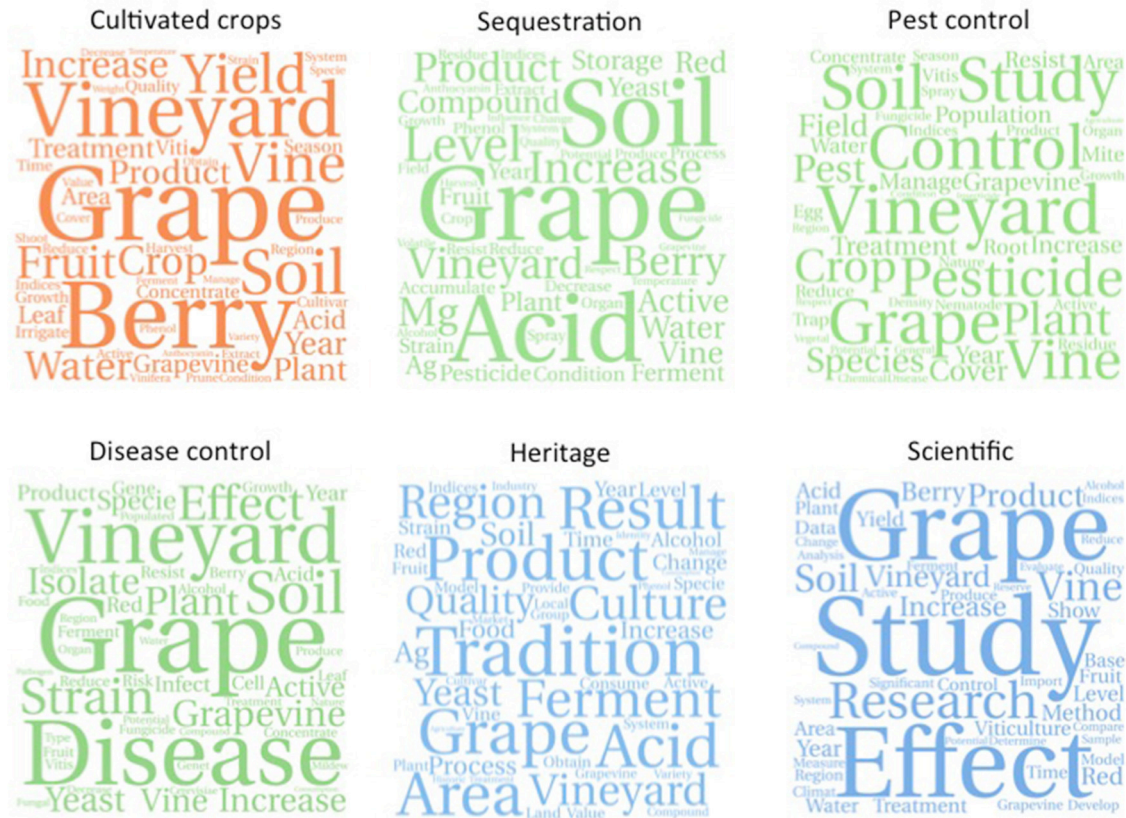
Cultural ecosystem services

Heritage

Ecosystems provide not only tangible services, but also non-material ecosystem services such as heritage and cultural traditions. These can be preserved in physical landscapes and also in historic records and traditional knowledge. In many wine-growing areas, such as Champagne, France, or Napa, California, vineyards are a dominant land use that characterize not only the local landscapes but also local cultural traditions, heritages and identities (Figure 4; Winkler and Nicholas, 2016). The wines as well as the vineyard landscape act as trademarks for the whole region (Daniel et al., 2012; Orre-Gordon et al., 2013). Especially the emphasis on wine production as part of the regional tradition can contribute to symbolic positions that are useful for marketing wine or the wine region (Beckert et al., 2014). The

United Nations Educational, Scientific and Cultural Organization (UNESCO) has designated multiple vineyard landscapes as World Heritage Sites, including Piedmont Vineyard Landscape in Italy and the terroirs of Burgundy in France (UNESCO, 2016).

Furthermore, beside the natural conditions such as soil composition and regional climate, *heritage and cultural* services in the form of the regional traditions of wine production contribute to terroir, the “taste of place” that many wine aficionados prize. Terroir reflects the unique aspects of a growing region with its typical winemaking traditions (Trubek, 2008). The existence of labels for protected appellation of origins, such as the Appellation d’Origine Contrôlée (AOC) in France and Switzerland, show the significance of heritage for the terroir of wines.



Scientific

climate adaptation, due to their high climate sensitivity as well as high potential for innovation and adaptation to climate change (Nicholas and Durham, 2012).

Objective 4: Relationships and Multifunctionality in Vineyards

Correlations among Services

We checked how many papers from the full Scopus literature review were found in more than one ecosystem service class. About 60% of the papers were found in at least two ecosystem service classes, and 32% were found in more than three ecosystem services classes. One explanation for the rather high numbers could be that some search terms were used for multiple ecosystem services. We used for example “landscape” as search term for services including *mediation of smell/noise/visual impacts, flood protection, aesthetic, and existence* (Table 1).

When looking at correlations among the publications identified for two single ecosystem service classes, we found that the single service classes most likely to appear in a publication with another service class were *cultivated crops* (28% of the papers that studied cultivated crops also studied another service) and *scientific* (22% overlap; **Table 3**, Supplementary Table 1). Not surprisingly this reflects the importance of crop production

TABLE 3 | Percent overlap between publications across the six key ecosystem service classes we identified in vineyards, with darker shading indicating greater overlap between the two ecosystem service classes.

In %	Cultivated crops	Sequestration	Pest control	Disease control	Scientific	Heritage
Cultivated crops		25	19	23	27	19
Sequestration	20		22	20	15	9
Pest control	17	24		22	9	5
Disease control	23	25	25		19	16
Scientific	25	17	9	17		23
Heritage	8	5	2	7	11	

Numbers indicate the percentage of the ecosystem services of the column that can also be found in the ecosystem service class of the row. For example, 19% of the publications identified through search terms for the ecosystem service class heritage were also found in the publications identified using search terms for the ecosystem service class cultivated crops, while only 8% of the publications for cultivated crops were found in heritage. Total amount of publications (raw hits) varies for each ecosystem services class (Table 1) and thus relative values vary also between ecosystem services classes.

in agricultural landscapes within our target of scientific publications. These numbers are a bit lower for other services within the six key services we identified (Table 3). Especially, publications found in *heritage* rarely study other ecosystem service classes. This seems to imply that research on *heritage* in vineyards pursues another research branch than the research that covers viticulture topics (e.g., *cultivated crops* or *disease control*).

Multifunctional landscapes provide multiple ecosystem services that are interlinked. Looking at vineyards, the six key ecosystem services are also connected (Figure 5). Much like the complexity of a fine wine and the human appreciation of its “bouquet,” the interplay of ecosystem services and the ability of science to identify and assess such linkages increases our understanding and appreciation. *Disease control* and *pest control* increase the yield of *cultivated crops* because only healthy plants can maximize productivity. The vines grown to produce the *cultivated crops* (grapes) also conduct photosynthesis, and thereby carbon *sequestration* in the vineyards. Lastly, winegrape production and the vineyard landscape foster a special cultural *heritage*. Without winegrapes and vineyards, large parts of the Mediterranean, and other global wine regions, would lose a key defining element of cultural identity. We have highlighted some of the most obvious relationships between the six key ecosystem services, but many other ecosystem services are highly connected, like *pollination* and *pest control* or *erosion control*, and *weathering processes*.

The six key ecosystem services we identified are provided at a range of scales (Figure 5). At a small scale, the vines themselves provide *cultivated crops* and *sequestration*. *Scientific* services are provided at the vineyard scale (e.g., studying vineyard agronomy), while *heritage* results from a combination of the vineyard and larger surrounding landscape scale. *Disease control* and *pest control* may be provided from habitat within vineyards as well as the surrounding habitat (e.g., hosting natural predators) at the landscape scale. This shows not only that most ecosystem services in a landscape are connected but also that a multifunctional landscape can provide ecosystem services at a range of scales that need to be considered.

DISCUSSION

We found a wide range of ecosystem services in vineyards. Nevertheless, a few services like *pest* and *disease control* as well as

the *cultivated crops* are in the center of interest: visible ecosystem services that influence the performance of the agrarian system and the marketable good produced. This does not mean that other ecosystem services do not exist or are of less importance for vineyard systems. Review studies on ecosystem services show that ecosystem services that are difficult to quantify are less often studied (Seppelt et al., 2011). Hence, there is a need for a greater acknowledgment of the range of services provided by ecosystems including vineyards.

In order to promote a multifunctional landscape, we must better capture a full range of ecosystem services and their connections. However, one of the current challenges in ecology is that there is only limited knowledge on these relationships, requiring future research (Bommarco et al., 2013; Birkhofer et al., 2015). A better scientific understanding of this multifunctionality can help to support maximizing the total benefit of a multitude of ecosystem services instead of maximizing one or two single ecosystem services at potentially high cost to others (e.g., Foley et al., 2005; Lovell et al., 2010).

A next step would be the consideration of how ecosystem management choices affect the provision of key ecosystem services. So far a broader perspective on a landscape, by e.g., adapting an ecosystem services approach, is often lacking (Bommarco et al., 2013). Based on the logic of Foley et al. (2005), adapting a land management strategy that aims to maximize a bundle of ecosystem services could promote a broad perspective on an agricultural system that will support a more long-term sustainable use of the land.

The ecosystem services concept can help to go beyond a narrow way of looking at the system and enlarge it by pointing out the multifunctionality of the land (Partelow and Winkler, 2016). The CICES classification includes 42 ecosystem services in three sections. In our case, we identified 27 of the 42 ecosystem service classes as relevant for vineyard systems. Our results indicate that ecosystem services in all three sections are studied, with a much stronger focus on provisioning and regulating and maintenance services than cultural services. The ecosystem services concept promotes inclusion for less studied or obvious services. The exercise of going through the whole list of ecosystem services to operationalize it in a specific ecosystem highlighted the multifunctionality of the system. This said, so far most publications on ecosystem services concentrate on one or two very specific ecosystem

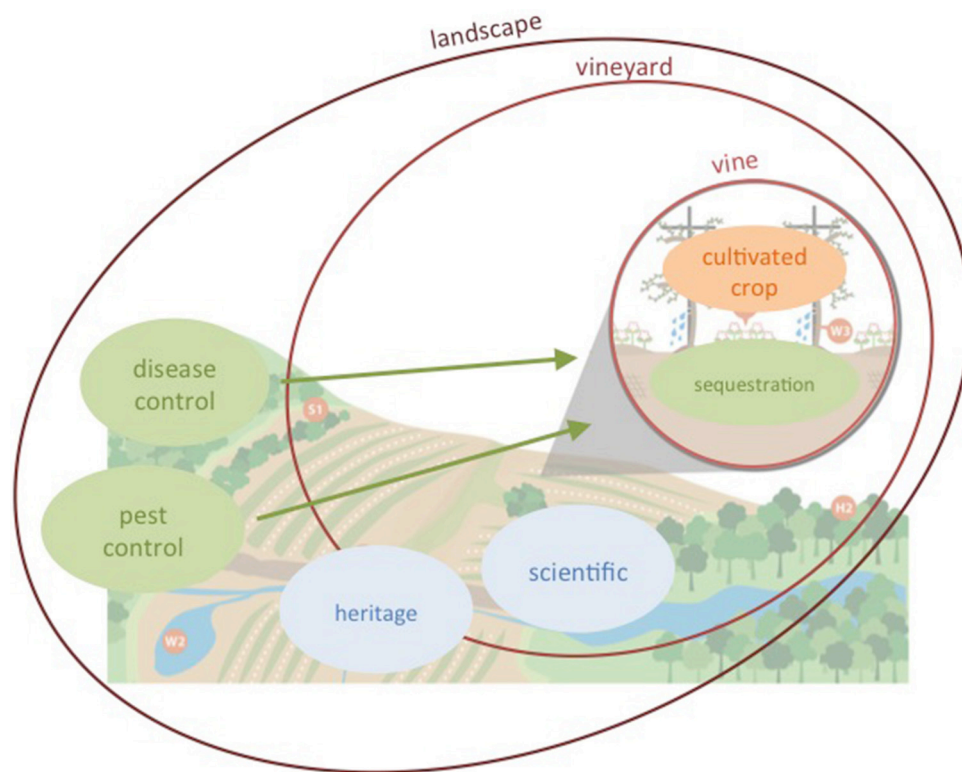


FIGURE 5 | Multifunctional vineyard system showing some relationships between the six key ecosystem services we identified (see text). The scale of ecosystem service provision varies from the vine, to the vineyard, to the surrounding vineyard landscape. (Underlying graphic from Viers et al., 2013; used with permission).

services (Seppelt et al., 2011) and many assessment methods can be criticized for their methodological narrowness (Silvertown, 2015). However, the research field is evolving: the use of broader valuation and assessment methods benefits the ecosystem services concept (Schröter and van Oudenhoven, 2016) and bundles of services are increasingly a topic of research (Queiroz et al., 2015; Renard et al., 2015; Mouchet et al., 2017).

Producers could use the ecosystem services approach to assess and promote the multifunctionality of their managed land and thereby choose to create multifunctional landscapes (TEEB, 2015; DeClerck et al., 2016). For vineyard systems specifically, they can adopt ideas like vinecology, which combines practices of ecology and viticulture (Viers et al., 2013): for example, creating wildlife habitat like hedgerows and vegetation strips in the vineyards to attract wildlife including pollinators, to enhance natural pest control, and to increase the aesthetic value to people (Jedlicka et al., 2011; Orre-Gordon et al., 2013). While voluntary actions in vinecology, such as set-asides for natural habitat and improved water management techniques, enhance and support ecosystem services, the economics to sustain such practices are often only realized when coupled with a robust market and targeted marketing strategies.

Limitations and Further Research

To our knowledge, this is the first effort to operationalize the CICES classification for a specific ecosystem (vineyard), and to do a systematic review if and how the resulting ecosystem services are researched. Other studies reviewing ecosystem services of one ecosystem did not stick to one classification, but rather merged different classifications schemes or stayed on the section level (e.g., provisioning, regulating, cultural; e.g., Liquete et al., 2013; Luederitz et al., 2015). We tried four different approaches to guide our search in order to meet the goal of discovering the full suite of relevant publications even if they did not use the specific term from the classification system.

While we believe this captures the most widely researched services, future studies could use a more methodically approach (see e.g., Schmidt et al., 2017). This goal could be achieved by e.g., using machine learning, or a broader and more formal expert elicitation, to select the search terms, to ensure the most relevant search terms are selected and avoid repetition of search terms between classes. This would help limit the subjectivity inherent in such an exercise of selecting search terms. In addition, we recommend considering balancing the number of search terms for each service studied, to avoid biasing the findings. A method comparison on the sensitivity of approaches for such a literature search would be useful to guide future efforts.

Despite these methodological limitations, we see great promise in operationalizing CICES to study specific ecosystems, as the comparability is a major purpose of the framework. Finally, this study can serve as a model for how one can do a first assessment of ecosystem services in a specific (agro) ecosystem. We hope our study can lay a base for future studies in order to be able to compare other production systems, and better study natural systems as well.

CONCLUSION

Our findings show that the ecosystem services concept has not often been used in research on vineyards. However, research exists on a plentitude of single ecosystem services, although it is typically not framed in the ecosystem services language. Vineyards are mainly considered as agrarian landscapes with a focus on visible ecosystem services such as *pest* and *disease control* and *grape production*. This limited focus is likely true of many agroecological systems, reducing our ability to manage for multifunctionality of the landscapes and thus for benefits for both people and nature. While it remains unclear why multifunctional studies have not been published more often, we believe that the ecosystem services approach could help scientists and practitioners to gain an understanding of the

multifunctionality of agricultural landscapes which in turn would help to promote more sustainable land management.

AUTHOR CONTRIBUTIONS

KW and KN designed the study. KW conducted the literature review. KW, KN, and JV wrote the article.

ACKNOWLEDGMENTS

We thank Rodd Kelsey and two reviewers for their comments on an earlier version that greatly helped to improve this paper. This research was partly conducted as part of the EU FP7-research project OPERAs (308393). KW is funded by the Ministry of Science and Culture of Lower Saxony (MWK) Research Training “Interdisciplinary approach to functional biodiversity research”(IBR) at Carl-von-Ossietzky-University, Oldenburg.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fenvs.2017.00015/full#supplementary-material>

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Conflict of Interest Statement: 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.

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High Nature Value Farmland: Assessment of Soil Organic Carbon in Europe

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High Nature Value Farmland (HNVF) is commonly associated with low intensity agricultural systems. HNVFs cover ~32% of the agricultural land in Europe and are of strategic importance for the European Union policy since they are reservoirs of biodiversity and provide several ecosystem services. Carbon sequestration is an important service that can be supplied by HNVFs as addressed in this study. Considering soil carbon content as a proxy for soil carbon storage, we compare HNVFs with soils that undergo more conventional land management (nHNVFs) and study the consequences of diverse land uses and geographic regions as additional explanatory variables. The results of our research show that, at the European level, organic carbon content is higher in HNVF than in nHNVF. However, this difference is strongly affected by the type of land use and the geographic region. Rather than seeing HNVF and nHNVF as two sharply distinct categories, as for carbon storage potential, we provide indications that the interplay between soil type (HNVF or nHNVF), land use, and geographic region determines carbon content in soils.

Keywords: ecosystem services, HNV farmland, land use, LUCAs dataset, soil carbon storage

OPEN ACCESS

Edited by:

Urs Feller,
University of Bern, Switzerland

Reviewed by:

Martin Potthoff,
Centre of Biodiversity and Sustainable
Land Use, Germany
Geoffrey R. Squire,
James Hutton Institute, UK

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 16 October 2015

Accepted: 08 June 2016

Published: 21 June 2016

Citation:

Gardi C, Visioli G, Conti FD, Scotti M,
Menta C and Bodini A (2016) High
Nature Value Farmland: Assessment
of Soil Organic Carbon in Europe.
Front. Environ. Sci. 4:47.
doi: 10.3389/fenvs.2016.00047

INTRODUCTION

The term High Nature Value Farmland (HNVF) was introduced in the early 1990s (Baldock et al., 1993; Beaufoy et al., 1994) to underline the relationship between agriculture and biodiversity (Trisorio and Borlizzi, 2011). Andersen et al. (2003, 2007) defined HNVFs as “those areas in Europe where agriculture is a major (usually the dominant) land use and where that agriculture supports, or is associated with, either a high species and habitat diversity or the presence of species of European conservation concern, or both.” HNVFs comprise small patches of semi-natural land covering the farmed landscape. Despite the little dimension, such patches effectively contribute to the conservation of biodiversity in agricultural areas (Beaufoy et al., 1994; Plieninger and Bieling, 2013). According to Lomba et al. (2014) landscapes of high value for nature conservation in Europe comprise: (1) farmlands under low intensity management, with a high proportion of associated semi-natural vegetation used as a forage or fodder resource; (2) farmlands characterized by low intensity management and mosaics of semi-natural and cultivated land; and (3) farmlands that provide habitat to one or more species that are rare either at the European or on a global scale. It has been demonstrated that HNVF is in general associated to low input agricultural systems (Pointereau et al., 2007). HNVF is envisaged as a way to maintain and improve biodiversity as well as to promote ecosystem restoration, bringing also socio-economic benefits (Peneva et al., 2014).

Ecosystem services (ES) depend on ecosystem functions and are beneficial to the human population (Daily et al., 1997; MEA, 2005; Lavelle et al., 2006). Among the key services, climate regulation is one of the most important both on global and European scale. Carbon sequestration is a fundamental process for this service to be effective and soils possess a high potential storage capability. They are the major reservoirs of terrestrial carbon with an estimated mass that amounts globally to $1200\text{--}1600 \times 10^9$ Mg of carbon (Eswaran et al., 1993; Batjes, 1996; Zech et al., 1997). Several studies aimed to estimate soil organic carbon (SOC) content within the ecosystem services framework (Egoh et al., 2008, 2009; Naidoo et al., 2008). Besides climate regulation, soil carbon is crucial for the maintenance of soil fertility and the prevention of erosion and desertification (Palm et al., 1997), therefore providing further ecosystem services on the local as well as the global scale (Marks et al., 2009).

Intensive agriculture, characterized by a massive use of inputs (fertilizers, herbicides, pesticides, etc.) and practices (tillage, different rotations per year, irrigation, etc.) to maximize harvest, has negative local, regional, and global consequences on above and below-ground biodiversity (Tsiafouli et al., 2015), on soil organic matter and thus on soil carbon content (Matson et al., 1997). Accordingly, it reduces the quality of soils (Karlen et al., 1997). In particular, carbon loss is clear in agroecosystems, but a wise management (e.g., no-till, cover crops, green, or animal manure) could reduce the rate and the amount of this loss (Matson et al., 1997; Naidoo et al., 2008). Thus, a central aspect of interest is to assess whether and how different land management types and forms of land use can affect the level of organic carbon in soils. This will allow the design and implementation of management strategies for carbon preservation in agricultural soils. In this study we address this matter by comparing the current carbon content of HN VF and nHN VF soils. The hypothesis we tested was the possible influence of land management (HN VF vs. nHN VF), and the interactions with land use/crop types and geographic regions on soil carbon content. In particular, we address the following questions: (a) is there a significant difference between HN VF and nHN VF in terms of carbon content? (b) Does the carbon content in HN VFs and nHN VFs depend on land use/crop types and geographical distribution of the soils? To answer these questions we exploited the LUCAS dataset, an extensive database that contains detailed information on land cover and land use across all European Union member states.

MATERIALS AND METHODS

Sampling and Soil Analyses

LUCAS (Land Use/Cover Area frame statistical Survey—EUROSTAT, 2015) is a survey created to perform a regular monitoring across all European Union member states. It contains information on land cover and land use that was gathered by direct field analysis of topsoil (Panagos et al., 2013). LUCAS was coordinated by the European Commission's Joint Research Centre in 2009 and 2011; field activities (survey and sampling) were carried out by professional consultancy companies operating at level of the member states. The purpose

of the survey was to collect soil samples in 23 member states of the European Union (EU), and analyze them to characterize the main chemical and physical features. Although LUCAS samples were taken from all types of land use and land cover, the agricultural areas were the most deeply investigated. Topsoil samples (0–20 cm), each consisting of five sub-samples, were collected from ~10% of the 265,000 LUCAS survey points, resulting into a total of ~20,000 samples. The selection of sampling points was designed to be representative of the agricultural land uses of the different countries using a stratified sampling scheme that took advantage of land use and terrain information. To obtain a harmonized dataset with pan-European coverage, all the 20,000 soil samples were analyzed in a sole ISO-certified laboratory. The soil parameters analyzed were: coarse fragments, particle size distribution, pH (CaCl₂), pH (H₂O), organic carbon, carbonate, phosphorus, total nitrogen, extractable potassium, cation exchange capacity, and multispectral properties. For the organic carbon analysis, the soil samples obtained after dry combustion at a temperature of 900°C were analyzed in a single ISO certified laboratory using the ISO 10,694 (ISO, 1995) method (Panagos et al., 2013; Tóth et al., 2013). The average density of LUCAS soil points was almost one sample every 200 km². An elevation comparison was used as additional indicator of representativeness of LUCAS soil point data. Elevation above 1000 m was considered a non-representative class. For more details about LUCAS data collection see Tóth et al. (2013) and Panagos et al. (2013).

European HN VF Map

We used a HN VF map that was compiled according to the methodology described in Paracchini et al. (2008). The compilation of such map comprises several steps. The first step for the selection of HN VF areas consisted of the overlay between the CORINE Land Cover (CLC 2000—EEA-ETC/TE, 2002), the Environmental Stratification of Europe Version 6 (Metzger et al., 2005; Jongman et al., 2006) and the country borders. This approach was adopted in the understanding that CLC class itself can be extremely different in terms of management and environmental characteristics across countries. For example, in Greece only the olive groves of Mediterranean Mountains (*sensu* Environmental Stratification of Europe) are included in HN VF; the same crop in Mediterranean South, Mediterranean North, or Alpine South is excluded. Another example is the case of rice fields: they are included in HN VF in Greece only, whereas in all the other countries they are excluded. This approach could probably be refined and improved by adopting the regional level (corresponding to the NUTS—Nomenclature of Territorial Units for Statistics—2) instead of the national level (NUTS 0), but the purpose of this study was to use the set of data as it was produced by the Joint Research Centre (JRC; Figure 1).

The selection criteria for HN VF (based on expert knowledge) are reported in the Annex II of Paracchini et al. (2008). In addition to the “*primarily HN VF land*” selected according to these criteria, relevant areas for nature conservation across Europe have been added. In particular, the following geographic datasets have been used: (1) NATURA 2000 network; (2) Important Bird Areas (IBAs); (3) Prime Butterfly Areas (PBAs; Van Swaay

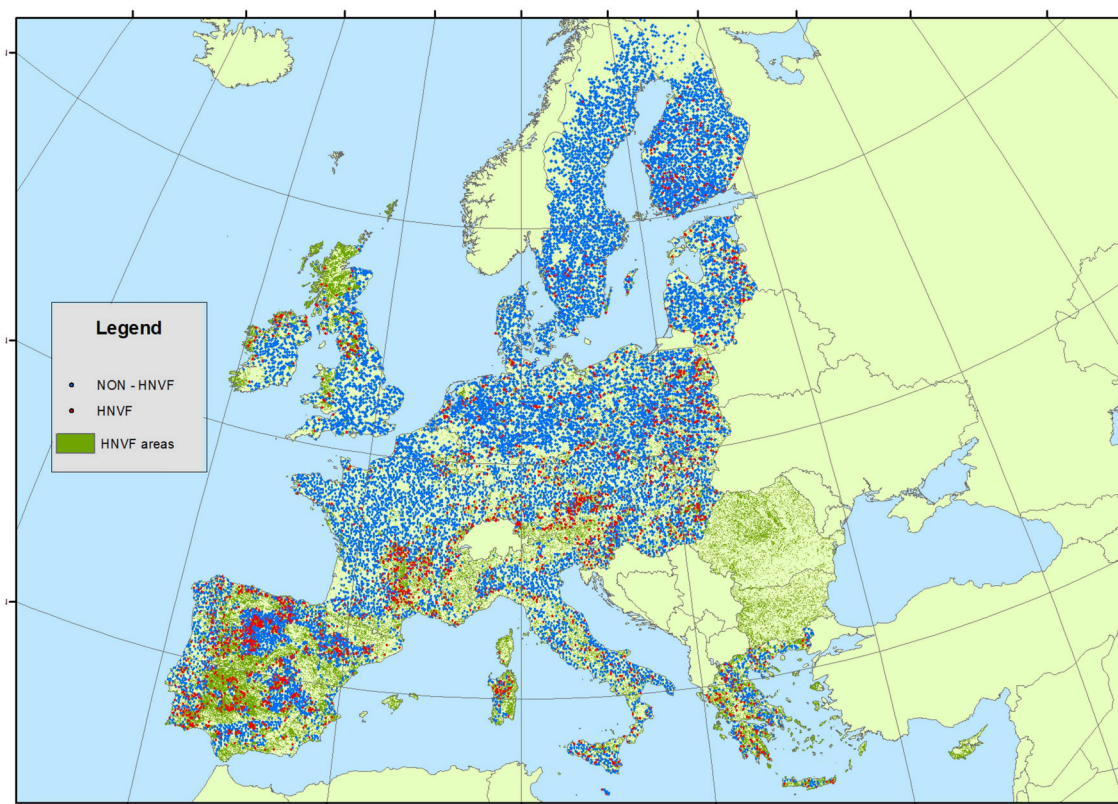


FIGURE 1 | Overlap of LUCAS soil sampling points over HNMF and nHNMF areas.

and Warren, 2003); and (4) national biodiversity datasets (when available). From these databases, agricultural areas relevant for biodiversity conservation but not detectable by the land cover approach have been included. From the national biodiversity datasets of some countries (i.e., Czech Republic, Sweden, Estonia, Lithuania, England) it was possible including in HNMF the semi-natural grasslands that otherwise would not have been identified from the Corine Land Cover map.

Data Selection and Analyses

In order to select and classify the LUCAS soil sampling points, based on their inclusion (or exclusion) within the HNMF areas, the two layers (LUCAS and HNMF) were overlaid using ArcGIS 10.2.

In order to explain the variation in SOC, we exploited the LUCAS dataset using a Generalized Linear Model (GLM) by carrying out regression analysis and ANOVA. Since our objective was to assess whether the organic carbon content in soils depends on the type of land management and whether this dependency may be influenced by the specific land use of the soils and by the geographic region where soils are located, we approached the statistical design considering that we had to test the response of a continuous variable (organic carbon content) to variations of three different predictors (nature of soil management, land use type, and geographic region). Our

main factors are: (1) the land management type, with two levels: HNMF and nHNMF; (2) the land use/crop type that includes 10 different levels corresponding to the agricultural practices of **Table 1**; and (3) the geographical distribution of the soils, with this latter predictor that consists of five levels corresponding to the geographical regions listed in **Table 2**. Crops are clustered into land use/crop type classes based on similar agronomic practices, while the division into geographical regions was essentially based on aggregation of countries according to the climate.

The form of the model we used is presented according to R syntax in the Supplementary Material (SM, henceforth). The model considers the organic carbon content as the dependent variable and the predictors are labeled as: (1) nature (HNMF vs. nHNMF); (2) land use; and (3) region. We used a GLM with Gamma family and log link. We selected the best model from all possible combinations of interactions between simple variables (with x that indicates the interaction between variables), guided by Akaike information criterion (AIC) statistics (Burnham and Anderson, 1998). We completed the analysis by performing a Tukey *post-hoc* test to highlight which differences are significant among all possible comparisons that include combinations of the three main factors. This test yielded as many as 6466 comparisons, an indicator of the level of complexity of the model.

TABLE 1 | Land use/crop types and corresponding classes in the LUCAS dataset.

Key	Type of land use/crop type	LUCAS classes	No. samples
WSC	Winter/spring cereals	(B11 B12 B13 B14 B15)	4353
SC	Summer cereals	(B16)	1095
OC	Oilseed crops	(B31 B32 B33)	376
RTC	Root/tuber crops	(B21 B22 B23)	755
VIC	Vegetable and industrial crops	(B41 B42 B43 B44 B45)	261
FC	Forage crops	(B51 B52 B53 B54 B55)	649
FB	Fruit and berries	(B71 B72 B73 B74 B75 B76 B77)	274
V	Vineyards	(B82)	420
O	Olive groves	(B81)	315
G	Grasslands	(E10 E20)	3935

Code for each one of the 10 classes (Key) and number of samples are indicated.

TABLE 2 | Geographical regions used to cluster EU countries.

Key	Geographic region	No. samples
CON	Continental Europe (Austria, Czech Republic, Germany, Hungary, Poland, Slovakia and Slovenia)	3802
ECO	Atlantic Europe (Belgium, France with latitude >44.00, Luxembourg, Netherlands, Portugal, Spain with latitude >42.50)	2806
BS	Baltic and Scandinavia (Denmark, Estonia, Finland, Latvia, Lithuania, and Sweden)	1470
MED	Mediterranean (Greece, Italy, Malta, Spain with latitude <42.50, France with latitude <44.00)	3352
UKI	UK and Ireland	1003

Number of samples per region and code (Key) are provided.

TABLE 3 | Summary of the ANOVA carried out with GLM.

	Df	Sum Sq	Mean Sq	F-value	p
Nature	1	187,928	187,928	134.22	<0.001
Land use	9	1,562,022	173,558	123.96	<0.001
Region	4	1,046,665	261,666	186.89	<0.001
Nature × land use	9	61,003	6778	4.84	<0.001
Nature × region	4	197,639	49,410	35.29	<0.001
Land use × region	30	192,462	6415	4.58	<0.001
Nature × land use × region	24	50,716	2113	1.51	0.053

The symbol x stands for an interaction between explanatory variables.

RESULTS

The outcomes of the ANOVA are summarized in **Table 3**.

From **Table 3** there is compelling evidence that all the three main factors are significant. So the level of organic carbon in European soils significantly depends on whether the nature of management is HN VF or nHN VF, what crop type they host (see **Table 1**), and where they are located within the geographic subdivision we considered (see **Table 2**). All two-way interactions are highly significant and the three-way interaction is significant at the 0.1 level of probability.

Table S1 presents all the differences in the mean values between the levels of the main factors and the related 95% confidence interval (CI). This output highlights that HN VF soils are significantly richer in carbon content than soils nHN VF

(nHN VF-HN VF = $-10.48 \text{ g SOC kg}^{-1}$, $p < 0.001$). The differences between the 10 levels that characterize the main factor land use were ascertained by performing multiple Tukey comparisons and as many as 17 out of 45 comparisons yielded significant differences (see Table S1). These significant results highlight that grassland soils are richer in carbon than soils hosting any other type of crop. This holds also for soils hosting forage crops: they store more carbon than any other crop except for grasslands. The differences between grasslands and other crop types, measured in g SOC kg^{-1} , are also higher than the differences observed between FC and the other crop types. All the other comparisons were not significant (Table S1). Nine out of 10 comparisons between geographic regions (Table S1) showed significant differences. A careful inspection of these outcomes reveals that soils (both HN VF and nHN VF) in United Kingdom and Ireland (UKI) contain significantly more carbon than soils in the other regions. On the contrary, soils in the Mediterranean region (MED) contain significantly less carbon than soils in any other region. Baltic and Scandinavian soils have more carbon than Atlantic Europe (ECO), Continental Europe (CON), and MED soils but less than UKI. Finally, it appears that no difference characterizes soils of the Continental Europe compared to soils of the Atlantic Europe. Moreover, we observed that the interactions between explanatory variables resulted significant. This means that the effect that each main factor has on the dependent variable (carbon in soils) is affected by the other factors as well. **Table 4** shows how the performance of HN VF soils in retaining carbon depends on the geographical region with differences in SOC between soils

TABLE 4 | Summary of the differences between the average amount of carbon in HN VF and nHN VF soils in the five geographical regions.

	Δ SOC	95% CI		<i>p</i>
	g kg ⁻¹	Lower	Upper	
(BS) nHN VF-HN VF	-10.87	-20.36	-1.38	0.011
(CON) nHN VF-HN VF	-15.15	-20.16	-10.14	<0.001
(UKI) nHN VF-HN VF	-68.29	-84.86	-51.73	<0.001
(MED) nHN VF-HN VF	-7.92	-12.91	-2.94	<0.001
(ECO) nHN VF-HN VF	-11.06	-17.34	-4.79	<0.001

nHN VF and soils HN VF that vary in magnitude within each region.

In all regions soils HN VF contain more carbon than nHN VF. The distributions of HN VF and nHN VF for the different regions are given as box plots in Figure S1. **Table 4** clearly shows that the difference between HN VF and nHN VF is much higher in UKI region than in the other areas (68.29 g SOC kg⁻¹). In the Mediterranean region this difference is less than 10 g SOC kg⁻¹ and in Baltic and Scandinavia the difference is less significant ($p = 0.011$).

If within all geographical regions the soils HN VF contain more carbon than nHN VF, when these two categories are compared considering each crop type across regions only grassland shows a significant difference in favor of HN VF (nHN VF-HN VF = -9.92 g SOC kg⁻¹, $p < 0.001$). Finally, the comparison regarded HN VF and nHN VF across regions and crop types (three way interactions). **Figure 2** helps to disentangle this information. It shows two charts for each region, one for HN VF and the other for nHN VF; each chart informs us about the distribution of organic carbon associated to the different crop types (land uses). For every single box plot we added the mean value and the standard deviation of the distribution it describes. Also, we depicted a common reference line that shows the HN VF overall mean value.

The results of the Tukey test are presented in Figure S2 in the form of upper triangular and squared color tables to facilitate the interpretation. They can be combined with **Figure 2** to understand the effects of three-way interactions. From **Figure 2** it appears that in all the regions, except for the Baltic and Scandinavia, grasslands contain more organic carbon than soils hosting other crops (in both HN VF and nHN VF). However, as Figure S2 illustrates, not all the contrasts in which grasslands are compared with other crop types for both HN VF and nHN VF soils yielded significant differences. This applies to all regions. In what follows we summarize some of the most noteworthy outcomes. In the UKI region grassland HN VFs store more organic carbon than grassland nHN VFs, and they contain more organic carbon than the soils hosting other crops. These latter are all of the type nHN VF since HN VF in UKI hosts only grasslands and some WSC. Also, grasslands in the UKI region are significantly richer in carbon than grasslands in other regions, as shown in **Table 5**. This table also shows that grassland nHN VFs in the UKI region contain more carbon than grassland HN VFs

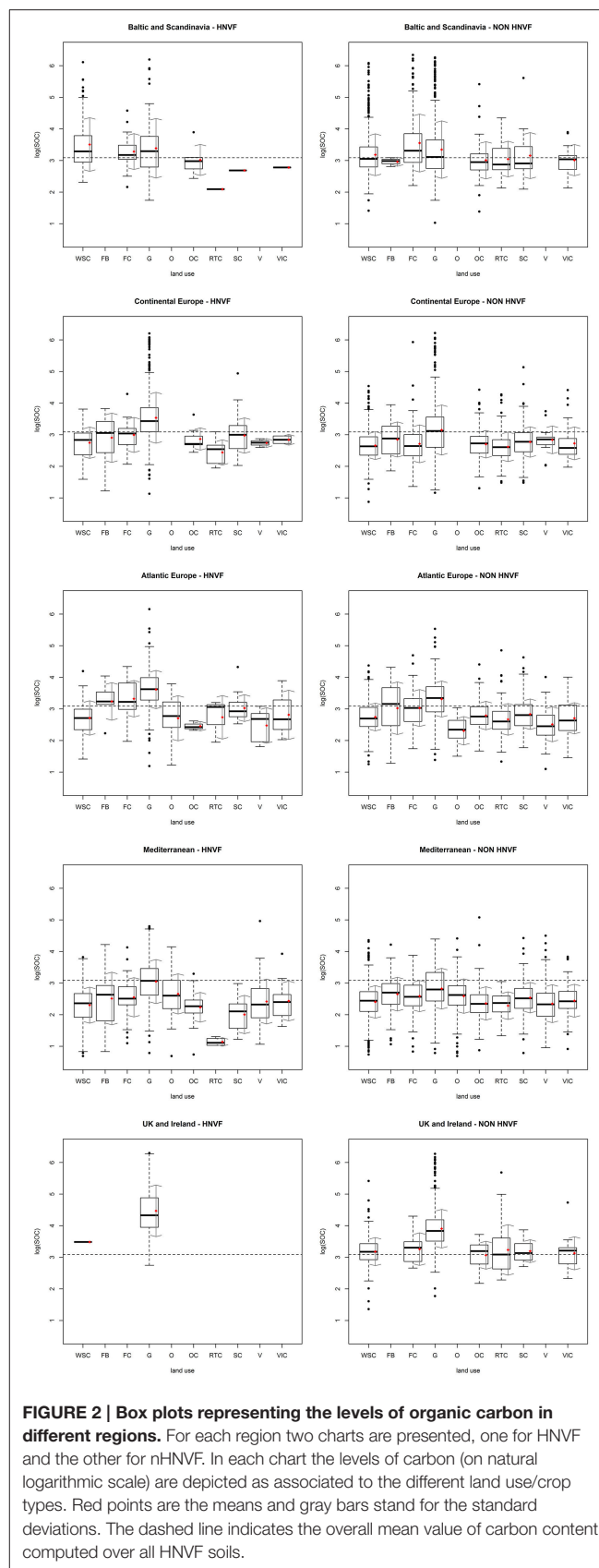


FIGURE 2 | Box plots representing the levels of organic carbon in different regions. For each region two charts are presented, one for HN VF and the other for nHN VF. In each chart the levels of carbon (on natural logarithmic scale) are depicted as associated to the different land use/crop types. Red points are the means and gray bars stand for the standard deviations. The dashed line indicates the overall mean value of carbon content computed over all HN VF soils.

TABLE 5 | Significant differences between grasslands UKI (both HNMF and nHNMF) and grassland HNMFs in the other regions.

	Δ SOC	95% CI		<i>p</i>
	g kg ⁻¹	Lower	Upper	
HNMF × G × UKI-HNMF × G × BS	74.52	46.06	102.96	<0.001
HNMF × G × UKI-HNMF × G × CON	74.67	51.40	97.93	<0.001
HNMF × G × UKI-HNMF × G × ECO	81.72	57.58	105.84	<0.001
HNMF × G × UKI-HNMF × G × MED	99.94	74.85	125.03	<0.001
nHNMF × G × UKI-HNMF × G × MED	36.36	22.72	49.98	<0.001
nHNMF × G × UKI-HNMF × G × UKI	-63.59	-86.71	-40.45	<0.001
nHNMF × G × UKI-HNMF × G × ECO	18.13	6.35	29.90	<0.001
nHNMF × G × UKI-HNMF × G × CON	11.08	1.21	20.95	0.005

The non-significant comparison between nHNMF UKI grasslands and grassland HNMFs of the Baltic and Scandinavia is not reported. The table also shows the difference between nHNMF and HNMF for grasslands in the UKI region (Δ SOC = -63.59 g kg⁻¹, *p* < 0.001).

TABLE 6 | Differences and 95% confidence intervals for comparisons between grassland HNMFs and other nHNMF soil types in the United Kingdom and Ireland.

	Δ SOC	95% CI		<i>p</i>
	g kg ⁻¹	Lower	Upper	
nHNMF × FC- HNMF × G	-97.06	-155.12	-39.00	<0.001
nHNMF × OC-HNMF × G	-103.34	-139.39	-67.28	<0.001
nHNMF × RTC-HNMF × G	-87.49	-124.28	-50.70	<0.001
nHNMF × SC-HNMF × G	-100.77	-152.25	-49.29	<0.001
nHNMF × VIC-HNMF × G	-99.77	-143.70	-55.83	<0.001
nHNMF × G-HNMF × G	-63.59	-86.72	-40.46	<0.001
nHNMF × WSC-HNMF × G	-99.88	-124.00	-75.75	<0.001

in the other regions. The only exception emerged when we compared grassland nHNMFs in the UKI region with grassland HNMFs of the Baltic and Scandinavia.

Within the UKI region the comparisons between grassland HNMFs and other nHNMF crop types yielded significant differences that are reported in **Table 6**.

In United Kingdom and Ireland HNMF grasslands show significantly higher carbon content than nHNMF soils (however, it should be noticed that only grasslands and winter spring cereals are present as HNMF in this region). Unlike the UKI region, the Atlantic Europe HNMF hosts a wide range of crop types. Nonetheless, the difference in soil carbon content between HNMF and nHNMF emerges as significant in the case of grassland HNMF only. The differences in the mean carbon content and the 95% confidence intervals for these comparisons are given in **Table 7**.

The grassland HNMF contains more carbon than most nHNMF crop types. Non-significant differences emerged, however, when the grassland HNMF was compared with forage crops (FC), fruits and berries (FB), and olive groves (O) nHNMF. Some of these results may appear unexpected, according to the box plots in **Figure 2**. For example, grassland HNMFs and olive grove nHNMFs are not significantly different (-21.26 g SOC kg⁻¹, *p* = 0.999). Looking at their distributions (**Figure 2**,

TABLE 7 | Differences and 95% confidence intervals for comparisons between grassland HNMFs and other nHNMF soil types in Atlantic Europe.

	Δ SOC	95% CI		<i>p</i>
	g kg ⁻¹	Lower	Upper	
nHNMF × WSC-HNMF × G	-27.83	-39.05	-16.61	<0.001
nHNMF × OC-HNMF × G	-26.95	-41.85	-12.05	<0.001
nHNMF × SC-HNMF × G	-25.77	-38.73	-12.80	<0.001
nHNMF × RTC-HNMF × G	-28.21	-45.64	-10.78	<0.001
nHNMF × V-HNMF × G	-30.63	-54.39	-6.87	<0.001
nHNMF × VIC-HNMF × G	-28.11	-50.43	-5.80	<0.001
nHNMF × G-HNMF × G	-12.78	-24.16	-1.40	0.005

Atlantic Europe panel) one would expect this difference to be substantial. The reason for this outcome is that the two samples are unbalanced and in these cases the Tukey test is conservative. In Continental Europe the grassland HNMFs show the highest content in organic carbon. Significant differences between HNMFs and nHNMFs emerged only when grassland nHNMFs were compared with nHNMFs, irrespective of the crop type. **Table 8** summarizes this evidence.

In the Baltic and Scandinavia region the comparison between HNMFs and nHNMFs yielded one significant difference only (i.e., between nHNMF OC and HNMF WSC: difference = -27.95 , *p* = 0.049). Grassland HNMFs seem not to hold the capacity to store significantly more carbon than other soils either in the HNMF and nHNMF categories, although the comparison of grassland HNMFs with WSC nHNMFs was close to the significance level (*p* = 0.054). The Mediterranean region is characterized by soils that tend to contain less carbon than the overall mean value of HNMFs (**Figure 2**, dashed line). This holds for both HNMFs and nHNMFs, although grassland HNMFs tend to reach that value. Grassland HNMFs contain significantly more carbon than WSC nHNMFs (difference = 14.16 g SOC kg⁻¹, *p* = 0.009).

Although HNMFs store more carbon than nHNMFs overall and in each and every single region (see **Table 4**), when the variability due to various land uses is incorporated this evidence becomes more heterogeneous across regions. In particular, the number of significant differences between HNMFs and nHNMFs, when associated with the various crop types, is higher in the UKI regions (seven significant comparisons), ECO (seven) and CON (eight). This is mainly due to the contribution of grassland HNMFs. All the significant differences between HNMFs and nHNMFs in these areas in fact emerge when HNMFs are in association with grasslands. In MED region as well as in the Baltic and Scandinavia only one significant comparison emerged between HNMFs and nHNMFs when considering the different land uses.

DISCUSSION

The HNMF concept introduced in the 1990s (Baldock et al., 1993; Beaufoy et al., 1994) was intended as an approach to differentiate the agricultural systems based on their contribution to nature conservation. It is one expression of the multifunctional

TABLE 8 | Differences and 95% confidence intervals for comparisons between grassland HNVFs and other nHNVF soil types in Continental Europe.

	Δ SOC	95% CI		<i>p</i>
	g kg ⁻¹	Lower	Upper	
nHNVF × WSC-HNVF × G	−36.30	−44.82	−27.78	<0.001
nHNVF × FC-HNVF × G	−32.17	−48.12	−16.22	<0.001
nHNVF × FB-HNVF × G	−32.05	−60.99	−3.11	0.007
nHNVF × G-HNVF × G	−16.82	−25.94	−7.70	<0.001
nHNVF × OC-HNVF × G	−35.66	−48.00	−23.32	<0.001
nHNVF × RTC-HNVF × G	−36.52	−51.75	−21.29	<0.001
nHNVF × SC-HNVF × G	−33.96	−44.64	−23.28	<0.001

role of agriculture, but also represents an indication of land use/land management intensity of agricultural systems. Land use intensity, within agricultural systems, is determined by the frequency and the intensity of anthropogenic activities, such as soil tillage, fertilization, irrigation, and pesticides application. Land management intensity can be estimated, for example, from the level of mechanization or specialization or the amount of inputs used (Donald et al., 2001). The hypothesis we tested was that soils in HNVF contain more organic carbon than nHNVF. In addition, we investigated how land use/crop types and geographic regions may affect this difference. The results of our analysis showed that, globally, the HNVF soils are characterized by higher organic carbon contents compared to nHNVFs. This evidence corroborates the fact that less intensively managed agroecosystems increase the potential of soils to accumulate carbon (Soussana et al., 2004; Rees et al., 2005; Grandy and Robertson, 2007; Ostle et al., 2009; Muñoz-Rojas et al., 2015) and deliver more ecosystem services (Björklund et al., 1999). The intensity of the management is generally related to the amount of inputs used, although not all inputs have the same effect on SOC storage. The intensity of tillage is generally inversely related to SOC storage (Govaerts et al., 2009), with no/minimum tillage systems storing more carbon than conventional tillage systems; however, other reviews showed the absence of significant differences between conventional and no-tillage systems. The effect of fertilization can be extremely variable. On grasslands nitrogen fertilization seems to reduce SOC content (Rees et al., 2005), while on arable lands inorganic fertilization generally increases the SOC content (Ludwig et al., 2010).

Our results indicate that also land use/crop type and the geographic regions are important factors influencing SOC content, confirming other scientific evidence (Xiao et al., 1997; Smith et al., 2005; Scharlemann et al., 2014; Lugato et al., 2015; Wiesmeier et al., 2015). The influence of these factors, which is highlighted by the results of the Tukey tests, is such that HNVFs and nHNVFs do not appear as sharply distinct categories as for carbon storage potential, but their performance is heavily affected by the other two factors. In particular, the pattern that seems to emerge is that HNVF shows significantly higher carbon content than nHNVF across regions when the land use

is grassland (see Figure S2). This holds with the exception of the Baltic and Scandinavia region. Several estimates of SOC densities and stocks at global scale have been published and showed a clear relation between latitude, climate, and SOC (Batjes, 1996; Scharlemann et al., 2014). On a global scale boreal moist and cool temperate moist are the climate types allowing to store more carbon in soil (Scharlemann et al., 2014). The differences between HNVFs and nHNVFs are larger in UK and Ireland, Continental Europe and Atlantic Europe, while in the Mediterranean, Scandinavia, and Baltic regions are less pronounced. The specific outcomes for UK and Ireland require further reflection. Grasslands in UKI region show the largest differences in SOC content between HNVFs and nHNVFs (63.6 g kg⁻¹). The majority of HNVF areas of the UK are in the Northern and Western Great Britain (mainly in Scotland and Wales) and in Ireland are located on the Atlantic west coast where the average rainfall is much higher than in the Southern and Eastern part (>1600 and <800 mm, respectively). It is demonstrated that wet and cold climates promote the accumulation of organic carbon in soil. Guo and Gifford (2002), in a meta-analysis on the effects of land use change on carbon storage, indicated that the highest potential for increasing SOC storage is associated with rainfall ranging between 1000 and 3000 mm per year. In addition, these areas are characterized by difficult terrain (e.g., steep slopes). Because of cold and wet climate, decomposition of organic matter is slower, while intrusive tillage is unlikely because of difficult terrain and because the climate makes tillage crops less profitable. These areas were classified as HNVF because they are marginal and in most of the cases low intensity is the only possible management. Soil characteristics and climatic factors associated to this “marginality” act in synergy with low intensity management to make SOC content high. Our results, combined with this evidence, recall the issue of the characterization of HNVF. In certain areas high carbon content and the classification of a soil as HNVF are not linked causally but co-occur because no other farmland is possible there. In the Mediterranean region soils tend to accumulate less carbon, irrespective of land management. This suggests that the potential to increase SOC content by land management practices is higher in Central Europe (Continental and Atlantic Europe) and UKI, compared to Southern or Northern Europe. This evidence is also confirmed by other researches on a European scale, such as the scientific and technical report of JRC on “Carbon Sink Enhancement in Soils of Europe” (Stolbovoy et al., 2007).

In general SOC content is the highest in grasslands. Also, the largest differences in SOC content between HNVFs and nHNVFs occur with grasslands (FC in BS region is the only exception). This confirms what previously documented in the literature (Scurlock and Hall, 1998; Soussana et al., 2004; Smith et al., 2005). In a recent modeling exercise at EU level, Lugato et al. (2015) posited that conversion of arable lands in grasslands is most effective in increasing carbon storage in agricultural soils. In the EU28 the area of permanent grasslands is 60 million ha and, on average, HNVFs cover 32% of the EU agricultural area. If we consider the possibility of increasing the percentage of HNVFs up to 50%, only for grasslands (+10.5 million ha) the benefit in

terms of carbon storage would be $\sim 0.4 \text{ Gt y}^{-1}$ (0–30 cm; bulk density 1.3 g cm^{-3}). Our analysis demonstrates that the HNMF management type can further increase the potential of grasslands in terms of carbon storage. The particularly high SOC content of permanent crops can be explained with the limited impact of soil tillage of these crops/land uses compared to the annual crops. It should be noticed, however, that recent researches showed that the role of no-tillage was often overestimated, while the potential of root apparatus as source of organic carbon inputs to the soil has often been underestimated (Giller et al., 2015). It is important to consider, however, that the classification/mapping exercise of HNMFs at continental scale is mainly able to detect differences on landscape level while very often, especially within the arable systems, the differences in management can be at the level of individual farms. In other words, the presence of an agro-ecological/organic/conservative farm within an intensively managed matrix/landscape cannot be detected and this is true also for the opposite case (i.e., an intensive farm within a less intensive landscape). This is particularly evident for arable systems where the different management options can play a major role at farm/field scale (conservation tillage, use of organic amendments, organic farming, etc.), while for grasslands the most relevant differences are operated at landscape/regional scale (grazing or non-grazing systems, agroforestry systems, etc.).

The peculiarity and added value of the results presented here is that they are produced based on a continental scale analysis. Such analysis has been carried out using a very large dataset and by comparing two land management systems. This research, however, also shows some limitations in the classification and mapping exercise of HNMF or, more in general, of agricultural systems at a continental scale. Indeed, at this level of spatial resolution it is not possible to detect variations in management practices that can have a relevant impact on SOC content and often occur at farm/field scale. Consequently, a more accurate evaluation of SOC content as function of different types of land/agricultural management should be done

at more detailed scale (i.e., watershed), replicated in different environmental conditions, and then up-scaled. Furthermore, it appears evident the intrinsic difference between the two datasets and the parameters/classifications used: while the measure of SOC content (or other soil parameters) is an objective and unambiguous metric, the classification of HNMF areas can be biased. HNMFs often are not the result of agricultural management deliberately adopted, but are the consequence of intrinsic limiting factors of the land.

AUTHOR CONTRIBUTIONS

CG experimental design, data extraction and analysis, literature review, manuscript preparation. CM literature review, manuscript preparation. GV literature review, manuscript preparation. FC literature review, manuscript preparation. AB statistical analysis, manuscript preparation. MS statistical analysis.

ACKNOWLEDGMENTS

We acknowledge the Joint Research Centre of the European Commission for providing the HNMF map of EU (M.L. Paracchini) and for the LUCAS Soil Dataset (L. Montanarella, G. Toth, A. Jones, and P. Panagos). This study was conducted as part of the LANDMARK (LAND Management: Assessment, Research, Knowledge Base) project. LANDMARK has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 635201. Dr. Jed Borowski is also acknowledged for language revision.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fenvs.2016.00047>

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- Conflict of Interest Statement:** 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.

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Establishing Wildflower Pollinator Habitats in Agricultural Farmland to Provide Multiple Ecosystem Services

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Keywords: pollinator, flower plantings, habitats, ecosystem services, agroecosystem, biocontrol

INTRODUCTION

Beneficial insects provide critical ecosystem services and in agriculture their contribution in pollination and pest control is widely evident (Losey and Vaughan, 2006; Kremen and Chaplin-Kramer, 2007). Globally, 35% of food production benefits from pollinator services (Klein et al., 2007). In many systems, pollination has been provided by the domesticated honey bee (primarily *Apis mellifera*), but the reliability of pollination services by wild pollinators is becoming increasingly valued (Garibaldi et al., 2013, 2014). These wild pollinators, the majority of which are bee species, persist independently in the ecosystem by relying on multiple resources to complete their lifecycles (Bohart, 1972). Similarly, natural enemies, such as insect predators and parasitoids, provide vital pest control and also persist independently in the farmscape. Although these beneficial insects are not directly managed for their ecosystem services, the farm landscape surrounding targeted crop fields can be modified to increase their abundance and diversity resulting in increased ecosystem services to support a sustainable agricultural system (Landis et al., 2000; Hannon and Sisk, 2009; Holzschuh et al., 2012).

Managing farmscapes for these wild beneficial insects is especially critical as insects are threatened by human-mediated landscape disturbances (Tscharntke et al., 2005). With wild bee populations in decline (Potts et al., 2010), there is increasing interest in managing for wild bees by incorporating pollinator habitat into farmland. The concept of setting aside land specifically for wildlife within a farmscape is not new (Baudry et al., 2000), however, the addition of wildflower plantings or saving natural wildflower areas is a specific strategy that can be adopted for its multi-functionality in supporting both pollinators and natural enemies. It is especially valuable in that it can be modified and designed to fit specific cropping systems, landscapes, and support the lifecycles of a community of unmanaged beneficial insects. Here we consider how these variables have been examined in recent pollinator habitat studies, and discuss additional considerations to optimize wildflower plantings to benefit multiple ecosystem services.

UNDERSTANDING THE POLLINATION NEEDS OF CROPS

Supplementary wildflower plantings (adjacent to target crops) function by attracting pollinators from the surrounding landscape to the farmscape and ideally to “spill over” to the crop to provide pollination services (Blitzer et al., 2012; Williams et al., 2015). The purpose of such landscape enhancements is primarily to provide additional nectar and pollen sources for the bee community. In some specific studies, wildflower plantings have been demonstrated as an effective practice for benefiting pollination by increasing crop production (Feltham et al., 2015).

The composition of the wildflower pollinator habitat in farmscapes should depend on the pollination requirements of the crop. One flower-based strategy that may inherently increase

OPEN ACCESS

Edited by:

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 29 December 2015

Accepted: 08 March 2016

Published: 24 March 2016

Citation:

Sidhu CS and Joshi NK (2016)
Establishing Wildflower Pollinator
Habitats in Agricultural Farmland to
Provide Multiple Ecosystem Services.
Front. Plant Sci. 7:363.
doi: 10.3389/fpls.2016.00363

pollinators in the farmscape are mass-flowering crops that attract pollinators to the area and may benefit a growing pollinator population by providing a pulse of resources (Le Féon et al., 2013). However, there is the concern that mass-flowering crops can dilute wild bee populations, or there could be competition between crop flowers and concurrently blooming wildflowers (Holzschuh et al., 2008). Also, after a one-time pulse, resources may not be available to support the bee community during the rest of the season.

In cases of mass-flowering crops, additional floral resources should be available and must compliment the crop to be available before and after the crop bloom to extend the full foraging season of the pollinator community (Menz et al., 2011). Timing of the target crop bloom must also be considered, where early, short blooming tree fruit pollinators may need more floral supplements than the pollinators of summer crops, such as annual vegetables and fruit, when a greater diversity of floral resources is available. Any mismatch in complimentary composition of wildflower availability and crop bloom period may not be effective in benefitting wild pollinator community associated with the crop (Ritz et al., 2013).

INFLUENCE OF FARM LANDSCAPE ON POLLINATOR HABITAT

Simultaneously, both the farmscape and larger landscape affect the effectiveness of the wildflower pollinator habitat. Wild bee pollination services may be most effective on small farms (Isaacs and Kirk, 2010), and large farms may not be able to completely rely on wild bees (Klein et al., 2012). Landscape context largely affects the wild bee community that is present, in that a depauperate system may not benefit from additional flowers because few wild bees are present, and a heterogeneous, resource-rich landscape may not benefit from any additional resources because resources are readily available to a diverse bee community, but a simple landscape with fragmented resources and isolated bee communities would benefit most from an enhancement (Tscharntke et al., 2012). To ensure an effective wildflower habitat, a general survey of the area could be conducted to assess the current beneficial insect community, plant diversity, and their relative abundance. Only with an existing beneficial community can the population be supported to increase ecosystem services.

RESOURCE REQUIREMENTS OF WILD POLLINATORS

Most pollinator habitats are focused on floral resource availability to various pollinators, however, these habitat areas may also provide nesting habitat for pollinators (Cane, 2001; Rands and Whitney, 2011). Pollinators move among habitats in the landscape for various resources (Mandelik et al., 2012), but as central-placed foragers, they have limited foraging ranges and nesting habitat must be located within range of crops that require pollination (Ricketts et al., 2008). Pollinator habitats should also include a diversity of appropriate nesting areas,

especially because nesting requirements vary greatly based on wild bee natural histories (Cane et al., 2007). Species respond to the landscape at different scales (Steffan-Dewenter et al., 2002; Tscharntke et al., 2005), and therefore, the food and nesting resources must be both spatially, and temporally available to support a robust and healthy ecosystem (Vaughan and Skinner, 2008), and bee populations (Williams and Kremen, 2007; Zurbuchen et al., 2010). Further, maintaining diversity of season-long floral resources in these habitats is essential to support the diversity of bees (Williams et al., 2015). Additionally, sustainable agroecosystems are generally supported by a diverse pollinator community, thus species-specific resources must suit the requirements of multiple species found in that ecosystem (Winfrey et al., 2011).

OPTIMIZING MULTIPLE ECOSYSTEM SERVICES FROM POLLINATOR HABITATS

Multi-functionality is key in promoting land use as pollinator habitat (Wratten et al., 2012), and it should be considered as an intentional step in establishing pollinator habitats, not just a secondary consideration. Several studies have documented that setting aside habitat is effective for supporting natural enemies for pest control (Landis et al., 2000; MacLeod et al., 2004; Gontijo et al., 2013), and in certain cases it can be effective within a year of implementation (Walton and Isaacs, 2011). Native wildflower species can also support natural enemy communities (Blaauw and Isaacs, 2015). For example, natural enemies such as parasitoid wasps (Patt et al., 1997), and flower flies (Ramsden et al., 2015) would benefit from floral resources that are available in the pollinator habitat, and flower flies may also provide additional pollinator services (Jauker and Wolters, 2008). Season-long flowering plants in pollinator habitats also attract other wildlife, and creating wildlife habitat in farmscapes may increase crop yields as reported in a recent study (Pywell et al., 2015). Optimization of these habitats by including diverse perennial flowering plants that attract other wildlife, particularly, beetles, butterflies, and birds (e.g., pollinator and insectivore) will likely increase the aesthetic value of the farm as well as resulting ecosystem services. Different habitat designs could be examined to further increase the multi-functionality, such as using flowers that are also nitrogen fixers, or modifying habitat to function like hedgerows to prevention of soil erosion and storm water infiltration in farmland (Burel, 1996). Overall design can be optimized to build resilience to disturbances in order to provide steady ecosystem services, which will contribute sustainable agricultural systems (Foley et al., 2005).

Biodiversity conservation of arthropods is another important benefit of pollinator habitats in farmscapes. These habitats may also be appropriate for protecting and conserving endangered arthropod species by providing them an appropriate ecological niche to reproduce and sustain populations. However, such benefits are yet to be documented. Recent field research suggests that the negative effects of pesticides on pollinators can also be mitigated by habitat and landscape that supports wild pollinators community in farmland (Otieno et al., 2015; Park et al., 2015),

therefore these pollinator habitats might also serve as potential buffer zones for beneficial species in intensive agriculture. However, such spillover benefits of these plantings would also depend on their location as well as pesticide programs of the farms.

BARRIERS IN IMPLEMENTATION: THINGS TO CONSIDER BEFORE ESTABLISHING POLLINATOR HABITATS IN FARMLAND

In implementing pollinator habitat in the agroecosystem there is the concern of removing land from production, and growers must consider the cost vs. benefits of such habitat, plus additional establishment, and maintenance costs (Landis et al., 2000; Blaauw and Isaacs, 2015). Including the added benefit of multi-functionality can increase the value of establishing wildflower habitat. There is the concern that setting aside habitat could also support greater pest populations, but the associated increased natural enemy population can be effective in suppressing pests (Lee and Heimpel, 2005). While these plantings may have potential to harbor pest population in farmland depending on crop type as well as regional pest problems, further research in this field is needed to better understand how pollinator enhancement plantings impact herbivore populations including various species of pests. Different pollinator habitat modifications and plant species composition could minimize on-farm pest populations, while benefiting pollinator community and other beneficial fauna. Supporting beneficial insects promotes a sustainable agroecosystem as well as ecological interaction among plant and insect species groups (Saunders et al., 2016).

There is also the consideration of time associated with such investments, as it may take several years before pollinator habitat takes effect (Blaauw and Isaacs, 2014a), but the use of different incentive programs, through government agencies such as the United States Department of Agriculture (USDA)—Natural Resources Conservation Service (NRCS) cost share for pollinator habitat creation and maintenance, or Agri-environmental schemes, can help to offset costs (Vaughan and Skinner, 2008; Joshi et al., 2011). In addition to the aforementioned factors, the successful implementation of these plantings could be significantly influenced by the layout and design of plantings and selection of an appropriate location (with optimum distance from the target crops) as well as long-term maintenance. Size of floral

plantings could influence pollinator abundance and diversity (Blaauw and Isaacs, 2014b), and it could be a barrier in adoption when growers have limited land availability in their farmland. After initial establishment (with the help of government subsidies or incentive programs), growers will need additional resources to maintain these wildflower pollinator plantings in their farmland, and in long-term, cost associated with the maintenance of these plantings could be a major hurdle in the successful adoption and establishment. However, increased awareness and promoting multi-functionality may increase acceptance and use by growers who will benefit from the multiple ecosystem services provided by the addition of pollinator habitat.

RECOMMENDATIONS

Most of the studies conducted on pollinator habitats suggest their importance for conserving pollinators (mainly bees) and their ecosystem service in farmland. Fewer studies have investigated the role of these plantings in supporting on-farm biological control and pest control services or other common benefits such as biodiversity conservation. However, in order to maximize the multiple ecosystem services from these habitats in farmland, it is important to examine ecological interactions among various species group, habitat design, and different trade-offs resulting from adoption of this farm practice in agricultural systems. Moreover, examining such interactions, design, and trade-offs will enhance our knowledge toward establishing robust and self-sustained pollinator habitats for sustainable agriculture. Therefore, a comprehensive life-cycle assessment of pollinator habitats and associated overall benefits could be considered as future research areas in this field.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

CSS is supported by UC Riverside, and NJ is supported by UA Fayetteville. Authors are thankful to the reviewers for their valuable inputs on a previous draft of this article. The views and opinion presented in this article are those of the authors.

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Conflict of Interest Statement: 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.

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Maintaining the Restriction on Neonicotinoids in the European Union – Benefits and Risks to Bees and Pollination Services

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Keywords: environmental risks, European policy, insecticides, non-target organisms, pest control, risk assessment

OPEN ACCESS

Edited by:

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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 19 July 2015

Accepted: 14 January 2016

Published: 03 February 2016

Citation:

Klatt BK, Rundlöf M and Smith HG
(2016) Maintaining the Restriction on
Neonicotinoids in the European
Union – Benefits and Risks to Bees
and Pollination Services.
Front. Ecol. Evol. 4:4.
doi: 10.3389/fevo.2016.00004

Globally, most crops are entomophilous (Klein et al., 2007), and thus yield is dependent on or at least benefits from pollination services. Wild bees are, together with honey bees, important providers of these crop pollination services (Garibaldi et al., 2013). Recent findings suggest, that bee pollination not only improves yield, but also the quality of agricultural products—including several aspects such as appearance, nutrient content, or shelf life (Klatt et al., 2014). Both, the amount and importance of entomophilous crops, are increasing (Lautenbach et al., 2012; Smith et al., 2015), thereby contributing to feeding an increasing world population (Godfray et al., 2010). However, bees in agricultural landscapes are exposed to several pressures. The use of pesticides, which is an integral part of contemporary agriculture, has among other factors been proposed to contribute to bee declines (Goulson et al., 2015), supposedly endangering pollination services (Chagnon et al., 2015; Stanley et al., 2015).

Since the early 90s, neonicotinoids have provided a powerful and increasingly used tool against insect pests in many crops, including those visited by bees and other pollinators (Elbert et al., 2008). Concerns about negative effects of neonicotinoids on bees recently resulted in a European Union-wide restriction on the use of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) as seed treatment in bee-attractive crops to allow time to clarify their potential environmental impacts (European Commission, 2013). Such a restriction beyond the re-assessment in 2015 (European Commission, 2013) will cause significant changes in pest control management (Hughes et al., 2014). While the debate in science, policy, and the public has focused on effects of neonicotinoid on bees, we argue that it is essential to also evaluate the consequences of alternative pest control strategies (Gray and Hammitt, 2000).

The extent to which neonicotinoids benefit yields is not clear (Goulson, 2013; Noleppa and Hahn, 2013; United States Environmental Protection Agency, 2014). However, as the active substance is systemic and thus becomes distributed to all tissues, neonicotinoids can particularly target herbivorous pest insects in treated crops (Elbert et al., 2008). Thus, unless consumers accept higher food prices, restrictions on the use of neonicotinoids will require farmers to find suitable alternatives.

Continued restriction of the neonicotinoids will most likely result in an increased use of other classes of insecticides. Organophosphates, carbamates, and pyrethroids dominated the insecticide market before the approval of imidacloprid in the early 1990s initiated the global dominance of neonicotinoids as seed treatments (Elbert et al., 2008). Although many organophosphates and carbamates are no longer approved for use following recent re-evaluations by the European Commission (European Commission – Pesticides Database, 2015), approved active ingredients from these two large insecticide classes

could nevertheless provide suitable alternatives to the restricted neonicotinoids: most organophosphates and many carbamates are also systemic (Sánchez-Bayo et al., 2013) and thus can potentially target a similar range of pests (Elbert et al., 2008). However, active substances from both classes suffer from pest resistance (Arthropod Pesticide Resistance Database, 2015). Pyrethroids are not systemic (Essential Chemical Industry online, 2015) and problems with pest resistance have been found (Heimbach and Müller, 2013; Arthropod Pesticide Resistance Database, 2015). Newer classes of insecticides such as, pyridin-azomethines (e.g., pymetrozine), phenylpyrazoles (e.g., fipronil) or the non-systemic, but plant-tissue infiltrating oxadiazines (e.g., indoxacarb) (European Commission – Pesticides Database, 2015) may be other potential alternatives. In addition, not all neonicotinoids are restricted (European Commission, 2013; European Commission – Pesticides Database, 2015). In particular acetamiprid and thiacloprid may be preferred alternatives in crops that are attractive to bees, as they also function systemically and are considered to be less acutely toxic to bees than their restricted and unrestricted (nitenpyram, dinotefuran) relatives (Blacqui re et al., 2012; European Commission, 2013).

Active substances from many insecticide classes that may replace the restricted neonicotinoids have already been shown to cause mortality in adults and larvae of honey bees, bumble bees and solitary bees (European Food Safety Authority, 2012; Arena and Sgolastra, 2014), and several are in fact considered as dangerous (B1; European Commission – Pesticides Database, 2015) or at least harmful for bees (B2; European Commission – Pesticides Database, 2015). The exposure to sublethal doses of neonicotinoids can impair the locomotive and cognitive abilities of bees (Blacqui re et al., 2012; Gill et al., 2012; Henry et al., 2012), however, such subtle effects have also been shown for bees exposed to sublethal doses of some alternative substances (European Food Safety Authority, 2012). However, as sublethal effects are currently not considered for bee-risk classifications (Cabrera et al., 2015), these potential impacts are largely unknown for most of the alternative substances and the risks these pose to bees under agronomically realistic conditions may remain undetected. For instance, although the neonicotinoids acetamiprid and thiacloprid can cause sublethal impairments (Blacqui re et al., 2012; Godfray et al., 2014, 2015; Brandt et al., 2016), with possible negative consequences for fitness under agronomically realistic conditions, they are still classified as not being dangerous for bees (B4) and can potentially be applied in flowering crops when bees are actively foraging (European Commission – Pesticides Database, 2015). Recent evaluations indicate that our knowledge about both possible risks and benefits of insecticide alternatives to restricted neonicotinoids remains incomplete (European Food Safety Authority, 2012). In addition, information on effects of insecticides is mostly available for honey bees, while there is a lack of knowledge of effects on wild bees and other important insect pollinators, particularly regarding long-term population consequences (European Food Safety Authority, 2012; Godfray et al., 2014, 2015).

In 2009 the European Parliament and Council developed a directive that farming within the European Union should follow

the integrated pest management principles (IPM) and contribute to sustainable use of pesticides (European Commission, 2009). The principles of IPM include the use of crop rotation and measures to promote pest natural enemies to control pests, as well as pest level monitoring and a threshold based use of insecticides (European Commission, 2009). This is expected to maintain the long-term efficacy of the insecticides, by reducing the development of resistance in pest populations (European Commission, 2009). However, since IPM depends on the use of insecticides as one of the pest control options (European Commission, 2009), it could therefore produce some of the same problems for beneficial insects that occur when implementing conventional pest management strategies, albeit at a smaller scale.

Besides IPM, the European strategy also considers plant breeding for pest resistance as an opportunity for pest control (Hartung and Schiemann, 2014). The cultivation of crop plants generated by genetic modification (GM) technologies could be an alternative to replace neonicotinoids, but the cultivation of GM crops is still constrained by stringent regulations in the European Union (Hartung and Schiemann, 2014). While the cultivation of plants from conventional breeding is less regulated, it is also less efficient in producing pest-resistant varieties than GM breeding technologies (Hartung and Schiemann, 2014), and may therefore not be an alternative to neonicotinoids in the near future. The most likely alternative to neonicotinoids available to farmers therefore is other insecticides.

Residues of many pesticides, including various insecticides, can be found in the hives of honey bees, with neonicotinoids constituting only a small proportion (Chauzat et al., 2011; Pettis et al., 2013). Hence, it is possible that risks with alternative pest management regimes following a restriction of neonicotinoids may continue to endanger bees and other pollinators, possibly replacing one threat with another. Posing such unpredictable consequences for pollination services could potentially impede future food supply.

Firstly, we conclude that our knowledge about the general impact of insecticides on pollinators is still limited (European Food Safety Authority, 2012). That an insecticide is toxic to individual bees or other pollinators is a trivial finding and the critical issue is whether bees in agricultural landscapes are affected in ways that may reduce the long-term persistence of populations (Cabrera et al., 2015) and in particular the pollination services they provide (Chagnon et al., 2015; Stanley et al., 2015). In addition, studies have been predominantly carried out using honey bees, disregarding that other bee species, as well as other pollinators, may differ in their sensitivity to insecticide exposure (European Food Safety Authority, 2012; Arena and Sgolastra, 2014). We argue that an expanded scientific evidence base is needed to assess the risks and benefits also of alternative pest management strategies.

Secondly, we conclude that current assessments of environmental risks with pest control methods have a limited ability to predict consequences for populations of different pollinator species under natural conditions. Our knowledge about direct and indirect effects of pest control on bees, in particular on bee populations under field conditions and

resulting consequences on pollination services is rudimentary, and our knowledge about effects on other pollinators is virtually non-existent. Reports of sublethal effects on bees leading to impaired locomotive and cognitive abilities (Blacquière et al., 2012; Gill et al., 2012; Henry et al., 2012), decreased reproductive success (Whitehorn et al., 2012; Rundlöf et al., 2015) and the insufficient provision of pollination services to crops (Stanley et al., 2015), call for the use of new approaches to such risk assessments. An alternative assessment would be measuring sublethal effects as endpoints after insecticide exposure, to inform environmental risk assessments and thereby potentially also regulatory decisions (European Food Safety Authority, 2012; Cabrera et al., 2015). Such consequences cannot easily be revealed in laboratory studies alone, because only field studies may reveal if such effects appear under agronomically realistic conditions (Cabrera et al., 2015) where bees have to work to collect their food and if this translates to fitness consequences (Mommarts et al., 2010). We argue that to allow informed

decisions a combination of laboratory, semi-field, and field studies is necessary (Cabrera et al., 2015), considering for example multiple routes of exposure as well as including both lethal and sublethal effects on both wild and managed bee species (European Food Safety Authority, 2012).

AUTHOR CONTRIBUTIONS

BKK conceived and led the project. All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This work was funded by FORMAS through the strong research environment SAPES and strategic research environment BECC. MR was funded by the Swedish Research Council (330-2014-6439).

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Conflict of Interest Statement: 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.

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Proximity to Woodland and Landscape Structure Drives Pollinator Visitation in Apple Orchard Ecosystem

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OPEN ACCESS

Edited by:

Evangelia G. Drakou,
University of West Brittany, France

Reviewed by:

Johan Ekroos,
Lund University, Sweden
Joseph Tzanopoulos,
University of Kent, UK

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authors.

Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 31 December 2015

Accepted: 31 March 2016

Published: 22 April 2016

Citation:

Joshi NK, Otieno M, Rajotte EG,
Fleischer SJ and Biddinger DJ (2016)
Proximity to Woodland and
Landscape Structure Drives Pollinator
Visitation in Apple Orchard
Ecosystem. *Front. Ecol. Evol.* 4:38.
doi: 10.3389/fevo.2016.00038

Landscapes of farms and adjacent areas are known to influence abundance of various arthropods such as pollinators in commercial agricultural ecosystems. In this context, we examined the effect of heterogeneous landscapes surrounding and including commercial apple orchards on pollinator visitation and foraging distance during bloom period from 2011 to 2013 in Pennsylvania. Our results showed that the frequency of feral honeybees and solitary bee visits within an apple orchard depends on the proximity of the orchard to an unmanaged habitat (primarily comprised of forest). At the landscape scale, we found that the Mean Proximity Index, the Largest Patch Index, and the Number of Patches positively correlated with the visitation rate of dominant bee taxa (*Apis mellifera*, *Bombus* spp., and solitary bees) visiting apple flowers at low spatial scales (up to 500 m around the orchards). The Mean Proximity Index at 500 m was related to bee visitation patterns, especially for solitary bees and *A. mellifera*. Bees in all our study sites preferred to forage in areas with large homogenous patches up to 500 m around an apple orchard. This effect can be attributed to the mass flowering of apples that formed the largest proportion of the 500 m spatial scale. The Number of Patches at 250 m spatial scale was positively correlated with bee visitation, especially *Bombus* spp., probably because these areas had more habitats and more resources required by these bees. We conclude that retaining unmanaged habitats closer to commercial apple orchards will maintain biodiversity within the landscapes and insure pollination services to apples.

Keywords: apple, bees, heterogeneous landscape, pollination, largest patch index, mean proximity index, ecosystem service

INTRODUCTION

Agricultural crop production relies heavily on insects to provide pollination services (Zhang et al., 2007; Potts et al., 2010). Among several species of insects, managed bees, mainly honey bees (*Apis mellifera*), and bumble bees (*Bombus* spp.), commonly make up for shortages in wild pollinators and feral honey bees that provide pollination services in various crops (Potts et al., 2010). Wild bees are crucial in pollination of several cultivated and wild flowering plants, and recent research has shown that they are efficient crop pollinators (Tepedino et al., 2007; Aebi et al., 2012; Christmann and Aw-Hassan, 2012; Garibaldi et al., 2013).

During the last few years, population decline in honey bees and other wild bees like bumble bees have been reported in North America and Europe (Gixti et al., 2009; Cameron et al., 2011), although similar trends have not been documented in other parts of the world such as Australia and Africa. Among several interacting stressors that have been reported in the recent past (Smith et al., 2013), agricultural intensification is thought to be one of the major causes of pollinator decline (Inouye, 2007; Marini et al., 2012) mainly through the destruction of nesting and foraging habitats, and frequent use of pesticides. Preserving natural and semi-natural habitats surrounding farmlands under commercial crop production may provide nesting and forage resources and buffer pollinators from adverse conditions. Such natural resource management strategy may increase the amounts of ecosystem services provided by pollinators, resulting in increased crop yields, and quality of produce (Holzschuh et al., 2012).

Apple (*Malus domestica* L., *Rosaceae*) is a major pollinator-dependent and high-value fruit crop which relies heavily on insect pollinators to set fruit (Tepedino et al., 2007; Garratt et al., 2014; Sheffield, 2014). In 2007, apples were grown on more than 145,765 hectares in the U.S. that produced 4.73 million tons (\$2.22 billion US) (see USDA¹ economic research service 2010). Like many other roseaceous tree fruits (for instance, cherries, plums, pears etc.), bloom in apple occurs in the early spring when most solitary bee species are inactive and some social species such as bumble bees have not had time to build colony membership. During this period apples produce a large number of blossoms, a phenomenon referred to as mass flowering (Westphal et al., 2003) which increases the demand for insects, mainly bees, to pollinate them.

Orchard management in commercial apple production is quite intense and may create unsuitable conditions for nesting bees. Vegetation between tree rows in the apple orchard is regularly mowed and herbicides are applied beneath trees to reduce competition for water and nutrients (Merwin, 2003), which may also affect plant species competition for floral visitors such as bees in the orchard landscape. The orchards are also sprayed pre-bloom with some common organophosphate and neonicotinoid insecticides and during bloom with many types of fungicides to control various insect pests and diseases (Hull et al., 2009; Penn State Extension, 2014). However, semi-natural habitats that surround these intensively managed commercial orchards offer refuge that may protect bees from on-farm disturbances while providing floral resources and nesting habitats (Wilson and Thomson, 1991; Javorek et al., 2002; Williams and Thomson, 2003; Winfree et al., 2007). Several studies have recently examined the effect of habitat around apple orchards or other crops in relation to wild bee visitation and the potential of *A. mellifera* to provide comparable pollination services in the absence of wild bees (e.g., Ricketts et al., 2008; Garibaldi et al., 2011, 2013; Kennedy et al., 2013). However, these studies have rarely considered apples grown in heterogeneous landscapes like

those found in the Mid-Atlantic region of the eastern United States. Such differences in landscape, weather conditions, and bee community composition makes it difficult to draw relevant conclusions from other systems and regions, and highlight the need for regional studies that are relevant and realistic for pollinator management. Additionally, these regional studies contribute to a comprehensive understanding of pollinator declines.

Proximity of crop fields to semi-natural vegetation is important in enhancing pollinator diversity and the level of ecosystem services provided by pollinators to crops (Karanja et al., 2010; Blitzer et al., 2012). However, effects of proximity to semi natural vegetation may vary with the landscape context (Steffan-Dewenter et al., 2002; Ricketts et al., 2008; Jha and Kremen, 2013). Other studies measure proximity to unmanaged land, (e.g., Bailey et al., 2014), but few consider the shape and size of those unmanaged land parcels or landscape contexts. Some studies suggest that the shape and size of unmanaged landscape parcels matter in determining the diversity and foraging activity of pollinators (Steffan-Dewenter et al., 2002; Otieno et al., 2011; Marini et al., 2012). The Mid-Atlantic region, specifically Pennsylvania provides an opportunity to evaluate the effects of proximity to natural or semi-natural habitat in heterogeneous landscapes. In this context, here we examine the effects of diverse landscapes surrounding commercial apple orchards on pollinator visitation over a 3 years period. We explore the following three questions: (1) Does proximity to natural woodland affect pollinator foraging distances into an orchard? (2) How does the surrounding landscape context affect pollinator visitation on apple flowers? (3) How do different pollinator species differ in their responses to landscape structure in and around apple orchards?

MATERIALS AND METHODS

This study was conducted in Adams County, Pennsylvania during apple bloom periods of April–May 2011, 2012, and 2013. Apples in this county are grown in orchards covering ca. 8903 hectares, and it is the leading tree fruit producing area in the state, making Pennsylvania the fourth leading apple producing state in the USA.

Description of Study Orchards

The orchards in the region are generally established in south facing slopes on well drained soils and surrounded by a heterogeneous landscape comprising forests (>50%), shrubs, field crops and developed land, which is a typical geography of the eastern Appalachian region (Egan and Mortensen, 2012).

For this study, we selected five commercial orchards with similar management programs (such as pesticide, herbicide, and fruit thinning practices) and which did not stock managed honey bees, bumble bees, or solitary bees for pollination. The orchards were mature at 15–20 years, on semi-dwarf rootstocks, with an average spacing of ~4.5 m between trees by ~6 m between rows (average of about 600–700 trees/ha). Within the Golden Delicious, York, and Honey Crisp varieties of each orchard, apple trees ($n = 120/\text{orchard}$, i.e., 600 trees from five orchards)

¹USDA (2012). *Economic Research Service: Apple statistics*. Available online at: <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1002> (Accessed April 2014).

were individually selected by distance from the edge closest to woodland toward the center of the orchard, marked and referenced with a GPS unit for sampling and bee observations. The distance gradients were same in each study orchard, and were 15, 35, 55, 100, and 200 m from the edge of the adjacent wooded area into the orchard.

Pollinator Visitation

Observations were made during optimal weather conditions [i.e., low wind speed (>3.5 m/s), temperature above 16°C , partly cloudy or bright overcast for bee flight (Supplementary Material S1). For each of the two sampling dates per orchard, all flowers on each tree were watched by two trained observers for 1 min between 1000 and 1400 h, during good weather conditions. Each observer stood on either side of the tree to capture all visitations to that side of the tree. Three main pollinator taxa were recorded—*A. mellifera*, *Bombus* spp., and solitary bees. In addition, syrphid flies were also counted, but were low in numbers across all study years. Immediately after the pollinator visit observations, we net-collected vouchers of all non-*Apis* bees for 30 min each at 15, 35, 55, 100, and 200 m from the edge of the adjacent wooded area into the orchard. These bee samples were placed in vials, labeled, and chilled in the field and frozen in the lab until they could be pinned, labeled, and identified. All bees were identified to species (see Supplementary Material S2).

Landscape Analysis

The surrounding landscape was assessed with the National Agricultural Statistical Service (NASS) map layer 2012 and groundtruthed in October 2013. The NASS land-use maps were 30 m raster dataset. The maps were first uploaded in ArcGIS 10.1 and buffer layers were created at increasing spatial scales. These buffers started from the edges of the wooded areas where they were adjacent to the orchards and extended out to 250, 500, and 1000 m to reflect the flight ranges of bees from the wooded adjacent areas that are generally nesting habitats of wild bees (Greenleaf et al., 2007; Zurbuchen et al., 2010). These buffers were then uploaded in Fragstats 4.0 and the metrics were generated at the landscape level for each scale (McGarigal et al., 2012; McGarigal, 2014). We assessed collinearity by using a correlation matrix to eliminate collinear metrics. The following landscape metrics were retained for further analyses: (i) number of patches; (ii) edge density; (iii) largest patch index; (iv) mean proximity index (v) landscape shape index; and (vi) contagion. A description of these metrics is available from http://www.umass.edu/landeco/teaching/landscape_ecology/schedule/chapter9_metrics.pdf (accessed November 2013) and in Supplementary Material S3.

DATA ANALYSES

Data from 120 individual trees (the sum from the two observers on a given sampling date) were averaged across the sampling dates within each year. We used simple linear regression models to determine the relationship between the number of bee visits and distance from the woodland into the apple orchards.

To test how landscape composition affects the apple pollinator community, we used redundancy analyses to assess the strengths of correlations between various landscape metrics and bee taxa (CANOCO 5.0, ter Braak and Šmilauer, 2012); we calculated the proportion of variance explained by an ordination of the metrics in the selected groups against all the response bee taxa selected (Pakeman, 2011), and then a forward selection technique to obtain a subset of landscape variables to model multivariate bee community composition (Legendre and Legendre, 1998).

For each year, the mean pollinator visitation per 2 min per tree per year were fitted to landscape factors using generalized linear mixed effects models (glmm) in lme4 library (Bates et al., 2011) using R (R Development Core Team, 2013). We used a log-link function to account for Poisson distribution in our data. We fitted the model with “site” as a random term to account for the variance in the response variables. The entire model was fitted with all the landscape variables as fixed factors first, then the model was progressively simplified by deleting fixed factors that caused a significant reduction in the Akaike Information Criterion (AIC), a measure of the quality of a statistical model useful in model selection, until a minimum adequate model was attained (Otieno et al., 2011). The minimum adequate model was taken to be a model from which the removal of any of the fixed factors caused an increase in the AIC (Crawley, 2007). To assess the significance of each predictor in the minimum adequate model, we used a log-likelihood ratio test (L-Ratio test). We used the same modeling structure to test for each pollinator taxa (*Bombus* spp., *A. mellifera*, and solitary bees) as a response variable. The L-Ratio test was done by applying a two-tier process: first we fitted the minimum adequate model with all predictors, then fitted a second model excluding the one predictor variable at a time. Each reduced model was compared to the minimum adequate model to determine the significance based on its Chi-square statistic and *p*-value. The response variables tested were mean visits per apple tree by *A. mellifera*, *Bombus* spp., and solitary bee taxa. We did not include syrphid flies due to very low frequency of observation.

RESULTS

A total of 2083 bees were observed over the 3 years of sampling (872, 628, and 584 bees in 2011, 2012, and 2013, respectively). We recorded 32 species: 26 species of solitary bees, 5 species of *Bombus*, and *A. mellifera* (the full list of bee species is appended as Supplementary Material S2). Wild honey bees (*A. mellifera*) were the most abundant visitors, accounting for 60% of the total visits recorded across the 3 years of sampling. Solitary bees accounted for 33% of the total visits, while *Bombus* spp. made only 7% of total visits.

Proximity to Natural Woodland and Foraging Distance

Most foraging activity was recorded closer to the natural woodland; lowest activity was recorded farther into the orchard. The proximity of an apple tree to woodland significantly affected distances that bees foraged into apple flowers in 2011 ($R^2 = 0.601$,

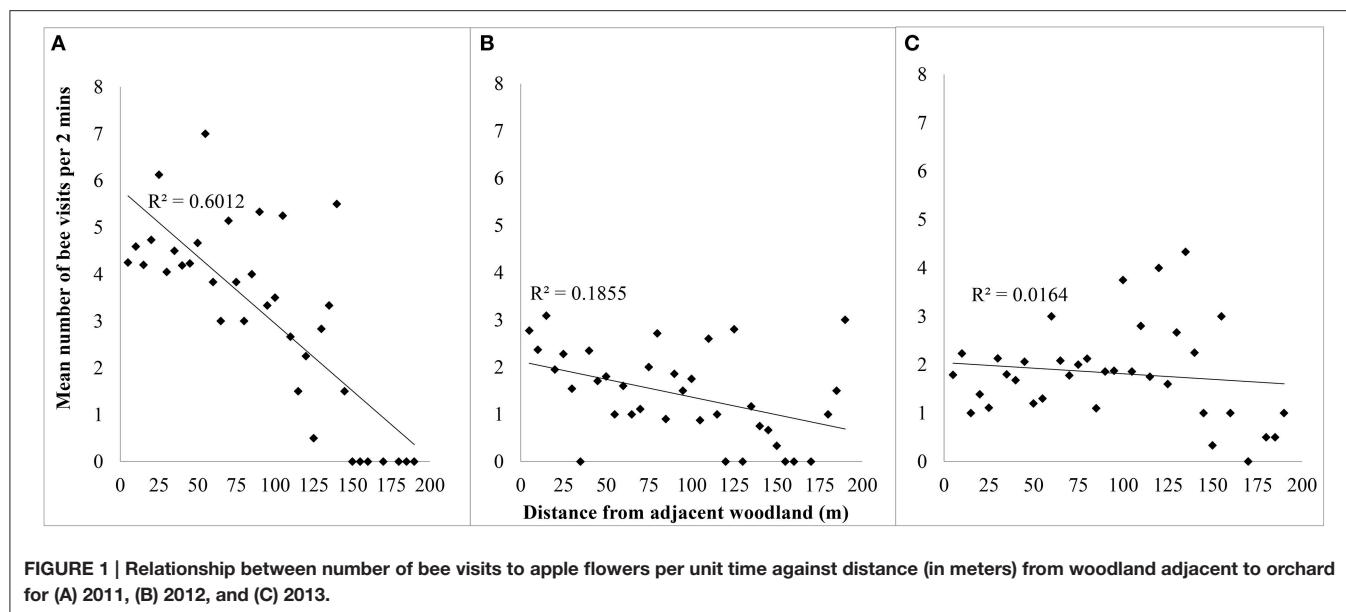


TABLE 1 | Summary of regression outputs of the relationship between bee taxa foraging on apple flowers (mean visits per 2 min count per tree per year) and distance (meters) from natural woodland.

Year	Bee taxa	Totals visits	R^2	P
2011	<i>Apis mellifera</i>	564	0.459	<0.0001
	<i>Bombus</i> spp.	59	0.217	0.005
	Solitary bees	248	0.617	<0.0001
2012	<i>Apis mellifera</i>	389	0.779	0.105
	<i>Bombus</i> spp.	23	0.293	0.326
	Solitary bees	216	0.45	<0.0001
2013	<i>Apis mellifera</i>	297	0.8	0.6101
	<i>Bombus</i> spp.	64	0.139	0.027
	Solitary bees	223	0	0.969

Solitary bee species list is provided in Supplementary Material S2.

$P < 0.001$), and 2012 ($R^2 = 0.186$, $P = 0.008$), but the effect was not significant in 2013 ($R^2 = 0.0164$, $P > 0.05$) (Figure 1).

Honey bees and solitary bees were responsible for the significant decline in foraging activity into the orchard from the woodland edge during the mass flowering period of apples in both 2011 and 2012 (Table 1, Figure 2). *Bombus* spp. contributed to the overall decline in foraging activity into the orchard in 2011, and were responsible for a slight and significant decline observed in 2013 ($P < 0.027$, Table 1). During 2013, *Apis* and solitary bee foraging activity did not significantly change with increasing distance from the orchard edge.

Impact of Landscape Context on Bee Visitation

Overall, the landscape at low spatial scales (250–500 m) significantly affected numbers of bee visits to apple flowers. The Mean Proximity Index at 500 m had the highest effect on

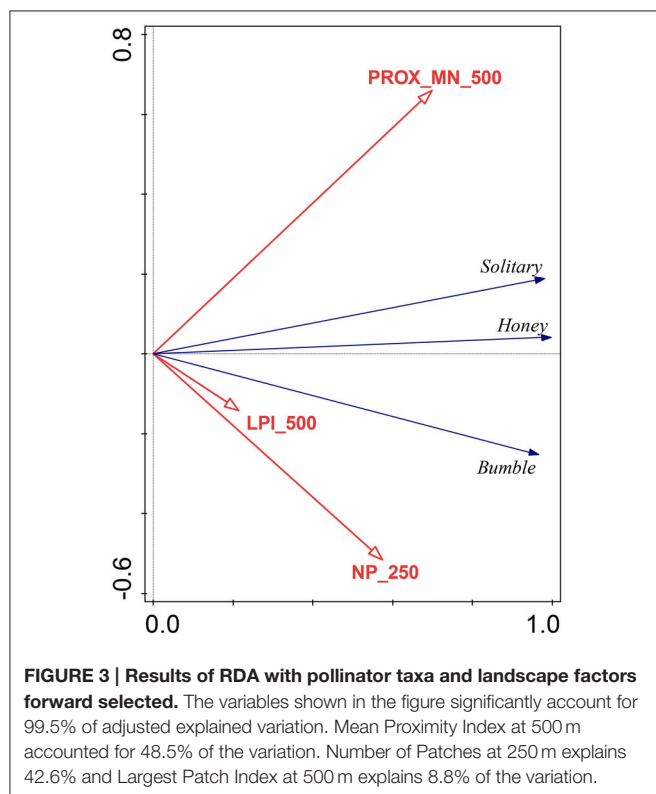
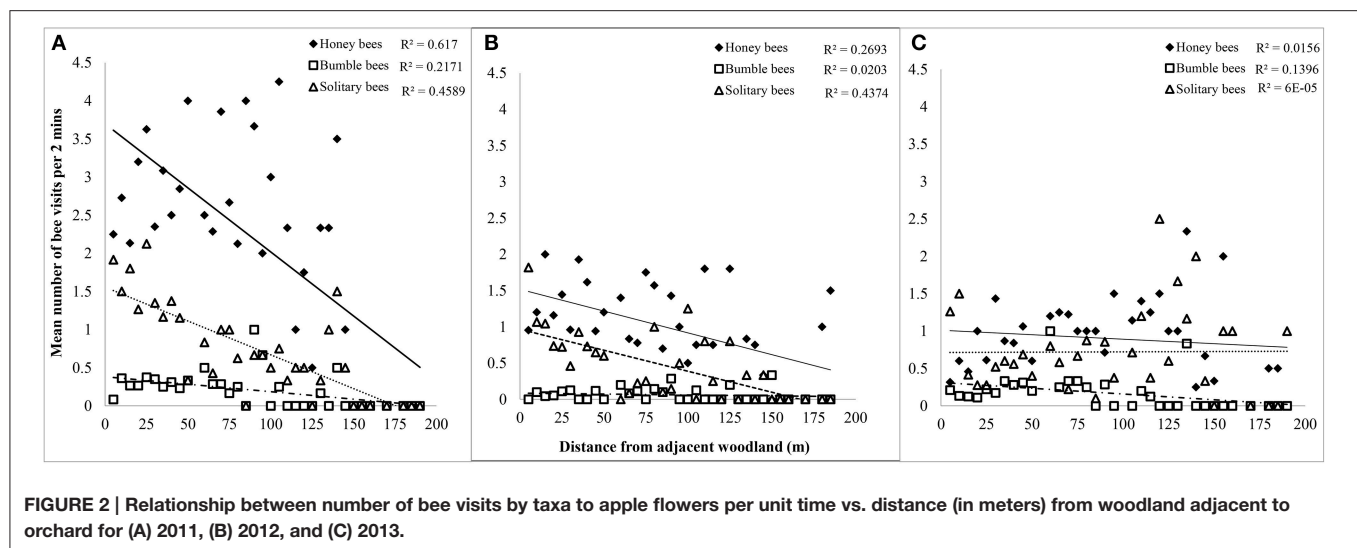
numbers of bee visits, explaining 48.5% of the total variation in the data (Figure 3).

The Number of Patches at 250 m and Largest Patch Index at 500 m explained 42.6 and 8.8% of the total explained variation, respectively. No other landscape factor significantly affected the number of pollinator visits. Solitary bees had a strong relationship with the Mean Proximity Index while *Bombus* spp. responded more to Largest Patch Index at 500 m and Number of Patches at 250 m in the RDA ordination. *A. mellifera* were largely unaffected, but showed a weak relationship with the Mean Proximity Index at 500 m (Figure 3).

Using a univariate linear mixed effects model approach to determine how the above landscape factors affected each bee taxa independent of the other taxa, we found all the bee taxa to be significantly affected by Number of Patches at 250 m, Largest Patch Index at 500 m, and Mean Proximity Index at 500 m (Table 2 and Figure 4).

DISCUSSION

Our study showed that the distance of apple orchards from surrounding heterogeneous landscape has significant effects on the foraging activity of bees that provide an important ecosystem service during bloom period. We also showed that landscape variables were more significant at much smaller spatial scales than previously reported (Kremen et al., 2004). Feral *A. mellifera* and solitary bee foragers were significantly more abundant closer to the natural woodland than they were in the center of the orchard. Bees prefer to forage in the most rewarding patches (in terms of quality and distance) in the landscape (Olsson et al., 2015), and the higher abundance of bees near natural woodland could be due to this reason. Similar to a previous study on bee community composition in apple orchards in this region (Joshi et al., 2015), we also observed high diversity of wild bees visiting



apple flowers during bloom period. These bee species, especially solitary bees, commonly live within natural, or semi-natural vegetation. Cavity-nesting bees have been shown to respond negatively to intense agriculture, presumably in response to loss of nesting habitat availability (Sheffield et al., 2013). The area bees forage is dependent on their nesting site as central-placed foragers. The distance they fly depends on their size and flight capability (Greenleaf et al., 2007; Zurbuchen et al., 2010), as well as floral resource availability (Biddinger et al., 2013). Presumably,

TABLE 2 | Linear mixed effects models showing L-Ratio (χ^2) outputs of the effects of landscape heterogeneity at varying scales on pollinator taxa visiting apple orchards.

Landscape factors	<i>Apis mellifera</i>		<i>Bombus</i> spp.		Solitary bees	
	L-Ratio (χ^2)	P	L-Ratio (χ^2)	P	L-Ratio (χ^2)	P-value
Number of patches at 250 m	21.782	< 0.0001	15.003	< 0.0001	19	< 0.0001
Largest patch index at 500 m	13.686	0.0002	8.542	0.0035	5.611	0.01789
Mean proximity index at 500 m	21.657	< 0.0001	5.398	0.0202	21.599	< 0.0001

if adequate food is available nearby, foragers will not fly beyond that distance.

Proximity to Natural Woodland and Foraging Distance

Generally, solitary bees can fly a few hundred meters, depending on availability of floral resources near their nests (Greenleaf et al., 2007). *A. mellifera* can forage much further (1100 m) from their nest (Gary et al., 1981) while *Bombus* spp. forage further away than most species (Greenleaf et al., 2007). Most bee species generally fly further from their nest if food (as floral resources) is in short supply, but fly a short distance to reach a plentiful supply of flowers (such as a mass-flowering apple crop) (Rao and Strange, 2012; Jha and Kremen, 2013; Sheffield, 2014). Pollinators may use natural and semi-natural habitats within agricultural landscapes as refugia. These refugia are important for bees and other insects because they provide alternative habitats for foraging, mating, and nesting (Westrich, 1996). As such, habitats such as woodlands adjacent to apple orchards may be an important source habitat of pollinators that forage on an apple crop (Watson et al., 2011).

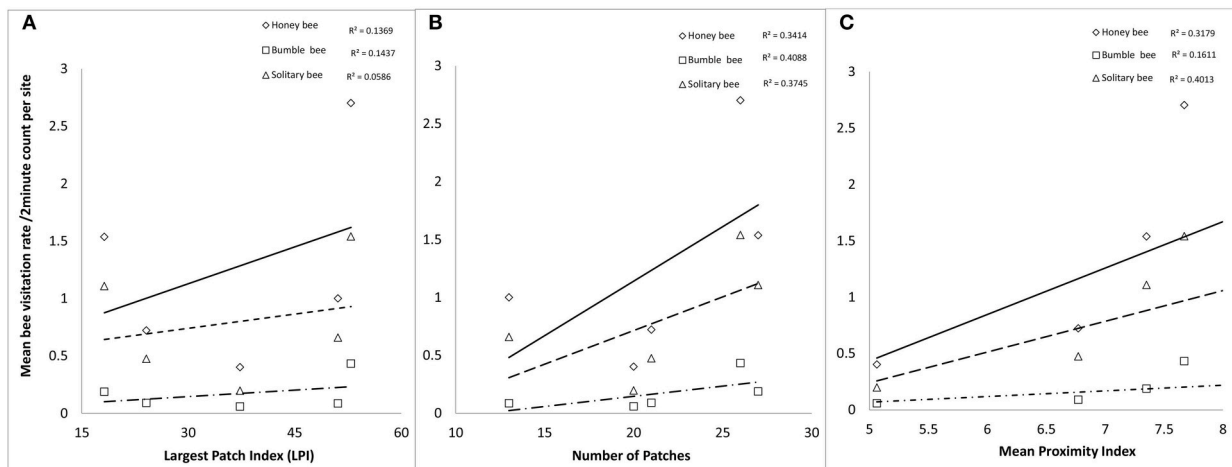


FIGURE 4 | Relationship between mean number of bee visits by taxa to apple flowers per unit time against (A) Largest Patch Index, (B) Number of Patches, and (C) Mean Proximity Index.

In ideal environmental conditions during apple bloom, pollinators constantly move between the natural habitats and crop fields (Garibaldi et al., 2011). Insects that migrate into crop fields to forage for pollen and nectar usually effect pollination in the process (Blitzer et al., 2012). Wild bee species that use semi-natural vegetation will move to crop fields when more resources become available within the crop (such as the mass-flowering apples) than in the natural vegetation (Blitzer et al., 2012). This phenomenon is illustrated in temperate and tropical agroecosystems. In the latter, wild bees from neighboring natural forest fragments increase yields and quality of coffee crops (Klein et al., 2003; Karanja et al., 2010).

Yearly variation in weather and bloom time affected overall bee foraging during our study. In 2011 we observed the highest number of bees foraging on apples. Sampling was done in bright, sunny and warm days and rain did not fall during this period. However, in 2012, spring occurred very early; flowers bloomed early and bees emerged early. Just before apple bloom, temperatures plummeted, and frost occurred (Supplementary Material S1). The bloom period was cold and rainy. Similarly in 2013 frost did not occur during bloom, but was dominated by overcast weather (Supplementary Material S1) and rain. These are not favorable conditions for bee flights (especially smaller solitary bees). Bees require optimal environmental conditions (temperature, humidity, wind) to effectively forage. At the time of apple bloom the only bumble bees we observed were queens in relatively low numbers, so we did not detect a significant association with semi-natural vegetation.

Impact of Landscape Context on Bee Visitation

Because bees have limited flight ranges from their nesting locations (Vicens and Bosch, 2000; Kremen et al., 2004; Biddinger et al., 2013), the landscape surrounding a farmland and the resources that the landscape provides, are important for our understanding of bee foraging patterns, bee conservation,

and ecosystem services provided by bees. Examination of the landscapes that surround a particular crop has interested landscape ecologists and conservation biologists in order to understand the patterns of bee visitation and pollination services (Kremen et al., 2004; Greenleaf and Kremen, 2006; Winfree et al., 2008; Klein et al., 2012; Cariveau et al., 2013). In this study, we found that landscape composition and configuration positively affected the dominant bee taxa (*A. mellifera*, *Bombus* spp., and solitary bees) visiting apple flowers at low spatial scales (up to 500 m around the orchards). Mean Proximity Index at 500 m, the Number of Patches at 250 m, and the Largest Patch Index at 500 m were the three main landscape metrics that correlated positively with bee visitation.

The Mean Proximity Index measures the connectivity of patches within the landscape; it takes into account patch size and distance to neighboring patches (Schweiger et al., 2010). In our study, landscape connectivity at 500 m was positively correlated with bee visitation patterns, especially for solitary bees and *A. mellifera*, but *Bombus* spp. visitation to flowers was very low (constituted only 7% of the total 2083 visits by all bees). Habitats that are highly interconnected, i.e., that have a higher Mean Proximity Index, have a better chance to retain high populations of bees and therefore increase pollination activity compared with habitats that are disconnected from one another.

The Largest Patch Index measures the percentage of the total area made up by the largest homogenous patch. Bees in all our study sites preferred to forage in areas with a large homogenous patch up to 500 m around a central point within an apple orchard. This effect can be attributed to the mass flowering of apples that formed the largest proportion of the 500 m spatial scale. In apple production system, large orchards (more than 10 ha per grower, which is $>100,000 \text{ m}^2$) could easily span beyond 500 m from an orchard's center. Apple bloom period is temporally partitioned so that the mass flowering occurs when few other wild plants or crops are flowering. Furthermore, in our landscape it is not uncommon for mass flowering to have occurred from

other rosaceous orchard crops (plum, peach, apricot) in close proximity to apple. However, they do not have much overlap in time. Thus, bees have the opportunity to move temporally among a series of mass flowering tree fruits.

The Number of Patches per unit area at 250 m was positively correlated with bee visitation. More bees, especially *Bombus* spp., responded to landscapes with more habitat patches, probably because these areas had more nutritional or nesting resources required by these bees. *Bombus* spp., *Apis* and some solitary bees are generalist foragers and may require additional resources beyond apple pollen and nectar to supplement their nutritional needs. In areas with multiple habitats, but within the vicinity of a large focal patch (i.e., the mass flowering apple crop), pollinators are moving across crop/non-crop interfaces. The largest diversity and abundance of pollinators is highest at the interface between crop fields and natural vegetation (the ecotone) because these habitats offer more floral and/or nesting resources compared to natural vegetation (e.g., forests, Hagen and Kraemer, 2010). Agricultural and other managed landscapes surely affect the pollinator-plant interactions of adjacent natural habitats.

Results of this study reveal that the effects of landscape configuration on bee visitation were more apparent at smaller spatial scales in apple production system. In general, the landscape in Pennsylvania and the other Mid-Atlantic States is quite heterogeneous. The Mid-Atlantic region is unique in that the landscape therein is a mosaic of different habitats and various land cover types are closer to each other, potentially providing season-long diverse floral resources required by pollinators, which explains the lack of significant results of landscape factors of bee visitation beyond 500 m of an orchard. This landscape structure supports the results of Winfree et al. (2007) with watermelon in the Mid-Atlantic region, who reported no effect of proximity to semi-natural habitats at a high spatial scale (>1000 m), and attributed it to the high heterogeneity of the Pennsylvania and New Jersey landscape. Similar patterns were reported by Lonsdorf et al. (2009) who used a predictive model to map pollination services based on pollinators nesting and floral resource availability and foraging ranges. Their model best fit areas dominated by highly intensive agriculture with low natural or semi-natural cover left (i.e., California and Costa Rica), but not our region (Pennsylvania and New Jersey). They attributed their results for this region to the small-scale heterogeneity defined by high plant diversity and interconnection between similar land cover types offering more nesting and floral resources than in highly intensified landscapes (Tscharntke et al., 2005). Bee foraging behavior in complex heterogeneous landscape (such as Pennsylvania apple orchards) may be more accurately predicted by the refined version of Lonsdorf model, which consider behavioral component into the model (Olsson et al., 2015). At larger spatial scales (e.g., more than 500 m), within heterogeneous landscapes, resources become more abundant and have marginal effects on pollinator visitation because the bees are already meeting their needs without having to forage far from their nests.

In regions with less heterogeneous landscapes such as California, for example, the abundance and diversity of bees strongly influenced by spatial characteristics of the landscape (Collinge, 2010). Farms that are surrounded by a low proportion

natural habitat cover (<1% within 1 km of the farm) has a low diversity and abundance of bees and low pollination of crops as a consequence (Collinge, 2010). Bees in such landscapes must fly further from their nests to meet their nutritional requirements. In such scenarios, they may be influenced by landscape context at much higher spatial scales than bees in heterogeneous settings such as Pennsylvania.

To conclude, our study revealed that the magnitude of feral *A. mellifera* and solitary bee foraging within an apple orchard depends on the proximity of the orchard to an unmanaged habitat such as natural woodland. For purposes of conservation, retention of unmanaged habitats near agricultural areas seems useful in maintaining biodiversity within the landscapes and therefore may help insure pollination. Furthermore, conserving unmanaged habitats in such landscape would also provide additional resources required by pollinators and other beneficial insects such as syrphids and other predatory flies, predatory beetles, spiders, and parasitoids, which in turn help to maintain a healthier community in and near the orchard. Economically, conserving natural areas near the apple orchard system and maintaining standard management practices that are safe to pollinators will ensure sustainable pollination and steady economic returns due to crops receiving sufficient pollination. Our results from landscape structure studies further support and complement the findings of the relationship between bee foraging activity during apple bloom period and the proximity of orchards to unmanaged habitats. We found landscape configuration at smaller spatial scales to be a significant positive determinant of bee visitation. Measures aimed at restoring natural pollinator visitation in and around apple orchards would be more successful if suitable habitats were provided at a smaller spatial scale (i.e., farm level) in heterogeneous landscapes such as Pennsylvania.

AUTHOR CONTRIBUTIONS

NJ, DB, ER, and MO conceived the study and designed the experiments. NJ, DB, and ER conducted field research and observations. MO collected landscape data and performed statistical analysis. SF contributed in data analysis and manuscript development strategy. MO, NJ, SF, DB, and ER wrote the manuscript. All authors reviewed the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge funding provided by the USDA-NIFA Specialty Crop Research Initiative, from project PEN04398: Determining the Role of and Limiting Factors Facing Native Pollinators in Assuring Quality Apple Production in Pennsylvania; a Model for the Mid-Atlantic Tree Fruit Industry, and from project 2012-51181-20105: Developing Sustainable Pollination Strategies for U.S. Specialty Crops, and the State Horticultural Association of Pennsylvania for conducting this research. Authors are thankful to K. Wholaver, K. Ellis, L. Miller, A. Ritz, J. Fissel for assisting in the fieldwork, E. Winzler for assistance in GIS mapping of field sites, R. Donovall, Dr. J. Gibbs

and Dr. R. Jean for helping with bee identification, and fruit growers: D. Slaybaugh, E. Diveley Jr., S. Slaybaugh and B. Knouse for allowing us to use their orchards to conduct this study. Authors are also thankful to K. Watrous for editing and C. S. Sidhu for reviewing an earlier draft of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fevo.2016.00038>

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Conflict of Interest Statement: 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.

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Using Whole-Genome Sequence Information to Foster Conservation Efforts for the European Dark Honey Bee, *Apis mellifera mellifera*

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OPEN ACCESS

Edited by:

Alberto Orgiazzi,
European Commission-Joint
Research Centre, Italy

Reviewed by:

Atanas Ivanov Atanassov,
Joint Genomic Center, Bulgaria
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 30 September 2016

Accepted: 05 December 2016

Published: 21 December 2016

Citation:

Parejo M, Wragg D, Gauthier L,
Vignal A, Neumann P and
Neuditschko M (2016) Using
Whole-Genome Sequence Information
to Foster Conservation Efforts for the
European Dark Honey Bee, *Apis
mellifera mellifera*.
Front. Ecol. Evol. 4:140.
doi: 10.3389/fevo.2016.00140

Pollination is a key ecosystem service for agricultural systems and Western honey bees, *Apis mellifera*, are the most important managed pollinators. Major losses of managed honey bee colonies reinforced the need to take advantage of locally adapted subspecies and ecotypes to buffer populations against various stressors. However, introductions of non-native honey bees from distant lineages are likely to undermine respective conservation efforts unless reliable and cost effective tools can be used to identify hybridization. The purpose of this study is to characterize current population structure and genetic diversity, and to assess the degree of admixture between native and introduced honey bees. Moreover, we aim to select a reduced number of genetic markers to improve conservation management strategies. We take advantage of recent developments in next-generation sequencing and network-based clustering to investigate conservation efforts for the native European Dark honey bee, *A. m. mellifera*, which is threatened by introgression in most of its range. We collected whole-genome sequence information from haploid drones of *A. m. mellifera*, *A. m. carnica*, and Buckfast sampled throughout Switzerland ($N = 81$), as well as from four Swiss *A. m. mellifera* conservation areas ($N = 39$) and from one conservatory in the French Alps ($N = 31$). Population structure analyses based upon 3.375 M genome-wide SNPs discerned samples by subspecies and geographic origin (Switzerland or France). Ancestry inference indicated admixed individuals in all of the protected areas, calling for improved management efforts. After testing different subsets of ancestry informative SNPs using three different selection strategies (F_{ST} , PCA-based or at random), as few as 50 SNPs are found to be sufficient to differentiate native from introduced honey bees. Therefore, our data suggests that a low-density SNP panel can be a precise and cost-effective tool to support conservation management efforts for managed pollinators.

Keywords: *Apis mellifera mellifera*, honey bee, conservation genomics, whole-genome sequencing, network clustering, admixture, ancestry informative SNPs

INTRODUCTION

Pollination is a key ecosystem service for agricultural systems (Klein et al., 2007) with the current annual market value of pollinator-dependent crops estimated to USD \$235–\$577 billion (IPBES, 2016). Global pollinator declines can have severe impacts on crop production and food security and calls for conservation of wild and managed pollinators (Potts et al., 2010; IPBES, 2016). The Western honey bee, *Apis mellifera*, is the most economically valuable pollinator (Klein et al., 2007; Gallai et al., 2009). Major losses of managed honey bee colonies across the Northern Hemisphere reinforced the need to take advantage of locally adapted subspecies and ecotypes to buffer populations against various stressors (Neumann and Carreck, 2010). The protection of honey bee biodiversity is therefore an imperative (De La Rúa et al., 2009), since current genetic diversity harbors the evolutionary potential of a species to adapt by natural selection in the future (Frankham et al., 2002; Allendorf et al., 2013). Once genetic variants are lost, they cannot be recovered and thus local adaptations to specific environments deserve conservation. In the case of the Western honey bee more than 27 subspecies have been reported, characterized by differences in morphology, physiology and behavior (Ruttner, 1988; Hepburn and Radloff, 1998; Sheppard and Meixner, 2003; Meixner et al., 2013; Chen et al., 2016, amongst others). These subspecies can be differentiated into four main evolutionary lineages: M (Western and Northern Europe), C (Eastern Europe), O (Near East and Central Asia), and A (Africa) (Ruttner, 1988; Garnery et al., 1992; Franck et al., 2000b; Whitfield et al., 2006; Han et al., 2012), whose geographic distribution encompasses a diversity of environmental conditions. The introduction of exotic subspecies from distant evolutionary lineages, typically by commercial beekeepers, poses a risk on the genetic integrity of locally adapted ecotypes (De La Rúa et al., 2009; Meixner et al., 2010; Pinto et al., 2014). It is thus essential to conserve the underlying genetic diversity, which may contribute to the long-term sustainability of populations (Vanengelsdorp and Meixner, 2010).

In Northern and Central Europe, the native dark honey bee, *A. m. mellifera*, has been widely replaced for beekeeping by subspecies mainly from the C-lineage such as *A. m. carnica* and *A. m. ligustica* (Ruttner, 1988; Moritz, 1991; Kauhausen-Keller and Keller, 1994; Jensen et al., 2005a), which have been more intensively managed for production and display more likely traits desired by beekeepers, such as high honey yield and docility (Bouga et al., 2011). The large mating distances of drones and queens (Ruttner and Ruttner, 1972; Böttcher, 1975; Jensen et al., 2005b), as well as the highly polyandrous mating system (Woyke, 1964; Adams et al., 1977; Neumann et al., 1999b) impose practical difficulties to conserve honey bee subspecies or to maintain breeding lines (Neumann et al., 1999a). This is further confounded by the ease at which different subspecies hybridize with one another (Franck et al., 2000a; Soland-Reckeweg et al., 2009).

In the last decade, there has been an increased awareness of the importance of preserving local honey bee subspecies (Muñoz and De La Rúa, 2012; Muñoz et al., 2014a,b; Uzunov et al., 2014b; Bertrand et al., 2015). A recent pan-European

experiment testing different subspecies and local hybrids across a variety of environments revealed that locally adapted bees were not only the most long-lived (Büchler et al., 2014), but in many cases also received better scores for docility and productivity (Uzunov et al., 2014a). Conservation efforts have been employed in many countries across Europe (De La Rúa et al., 2009) and typically focus on selective breeding or restricting the bees kept by apiculturists to those native to a specified area. Islands or remote mountain valleys in particular offer excellent opportunities to limit the impact of non-native honey bees. Conservation measures usually include that introduced honey bees and hybrids are replaced by native bees after being identified by discrimination of wing morphology (Ruttner, 1988; Kauhausen-Keller and Keller, 1994) or genetic analyses using microsatellite markers (Neumann et al., 1999a; Scharpenberg et al., 2006; Soland-Reckeweg et al., 2009; Oleksa et al., 2011; Péntek-Zakar et al., 2015).

In Switzerland, the native honey bee subspecies are *A. m. mellifera*, and to a lesser extent in Ticino, South of the Alps, *A. m. ligustica* (Ruttner, 1988). Since the middle of the last century, foreign honey bee queens have been increasingly imported such that nowadays two other subspecies or breeds are commonly found, namely *A. m. carnica* and the Buckfast bee (a highly selected hybrid breed). The persistence of *A. m. ligustica* in the region of Ticino is not clear. Many foreign queens have been introduced to this region and there are no ongoing efforts to maintain this local subspecies. In contrast, considerable effort has been invested to protect *A. m. mellifera* in Switzerland. To date, four conservation areas for *A. m. mellifera* have been established; conservatory Glarus (CGL) (~1000 colonies, 680 km²), Val Mustair (CVM) (~300 colonies, 199 km²), Diemtigtal (CDI) (~300 colonies, 135 km²) and Melchtal (CME) (~50 colonies, 150 km²). These areas are typically part of a nature reserve, and bee keeping of *A. m. mellifera* is either legally enforced or mutually agreed upon by the beekeeping community in that area. A national project to maintain and support these conservatories has recently been approved by the Swiss ministry of agriculture. However, for the effectiveness and long-term success of these conservation areas an accurate assessment of the admixture levels and genetic diversity of the current breeding populations is required.

In the French Alps, the center for technical bee keeping studies (CETA) was established in Savoie in 1997 to manage and select for the locally-adapted *A. m. mellifera*. At CETA de Savoie selection is based on biometric and morphometric analyses in collaboration with the French National Center for Scientific Research (CNRS) and Natural History Museum in Paris. The center shares the same objectives as the dark bee conservatories in Switzerland, and the region has comparable topography and climate. Analysis of bees from the different programs provides some information on the effectiveness of conservation management in the different regions.

The release and subsequent upgrade of the honey bee genome (Weinstock et al., 2006; Elisk et al., 2014) and rapid innovations in high-throughput technologies drastically reduced the costs of next-generation sequencing (NGS). Hence, it has now become both technically and economically feasible to assess genome-wide

genetic diversity and admixture levels of honey bees (e.g., Harpur et al., 2014; Pinto et al., 2014; Wallberg et al., 2014). However, despite the reduced costs of NGS it is still not cost-effective to routinely sequence hundreds or thousands of bees to monitor conservation management. Instead, a few but highly informative single nucleotide polymorphisms (SNPs), so-called ancestry informative markers (AIMs) can be selected in order to infer population structure (Shriver et al., 2003; Enoch et al., 2006; Kosoy et al., 2009). The identification of AIMs allows to estimate individual origin and admixture levels inexpensively and with great accuracy. For instance, Muñoz et al. (2015) have selected different AIM panels out of 1183 genotyped SNPs to examine levels of admixture between native *A. m. mellifera* and introduced honey bees in Europe. With such ultra-low density SNP-chips, thousands of individuals can be cost-effectively genotyped for conservation management or for sustainable bee breeding purposes. Moreover, SNP chips are potentially more accurate than the currently employed microsatellites or morphometric analyses for discriminating honey bees of different origin. Indeed, empirical comparisons for other species have shown SNPs to be more precise than microsatellites for population assignment and admixture estimation (Liu et al., 2005; Hauser et al., 2011; Gärke et al., 2012).

The purpose of this study was (1) to characterize the current population structure and genetic diversity of the honey bees sampled in Switzerland and the French Alps, (2) to assess the degree of admixture from introduced honey bees in the conservation areas of *A. m. mellifera* and (3) to select a reduced number of informative SNPs to improve conservation management strategies. To this end, we sequenced 151 whole-genomes of haploid drones sampled throughout Switzerland and the French Alps which included *A. m. mellifera* samples from five conservation areas. Using model- and network-based clustering approaches, we detected fine-scale population structure with high genetic diversity in all sampled subpopulations. Furthermore, we observed admixed *A. m. mellifera* individuals in all five conservation areas. To cost-effectively identify such hybrids within *A. m. mellifera*, we selected ancestry informative SNPs and show that as few as 50 SNPs are accurate to quantify levels of genetic admixture and relatedness between honey bees.

MATERIALS AND METHODS

Sampling, DNA Extraction and Sequencing

In summer 2014, sealed drone brood was sampled from 120 honey bee colonies at 87 apiaries throughout Switzerland (Figure 1). The beekeepers involved specified these samples to consist of 72 *A. m. mellifera*, 34 *A. m. carnica*, and 14 Buckfast. *A. m. mellifera* samples from Switzerland included 39 from four conservation areas: CDI ($N = 6$), CGL ($N = 17$), CME ($N = 6$), CVM ($N = 10$) (Figure 1). Drones were sampled because they are haploid allowing to confidently identify SNPs with less coverage than in diploid individuals (Wragg et al., 2016). Furthermore, the collection of drone brood allows excluding sampling errors due to drifting from neighboring colonies (Neumann et al., 2000).

In order to ensure high quality DNA for whole-genome sequencing, a two-step procedure was applied for DNA

extraction. First, high molecular weight DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) (Ausubel, 1988) from one entire honey bee drone per colony at the larval or pupal stage. DNA was then purified using the QIAGEN's EZ1[®] DNA Tissue Kit (QIAGEN Redwood City, www.qiagen.com). Pair-end (2×125 bp) libraries were prepared following the manufacturer's protocol (TruSeq Nano Kit v4) and whole-genome sequencing at an aimed sequencing depth of 10X coverage was performed on an Illumina HiSeq2500 with 24 samples per lane.

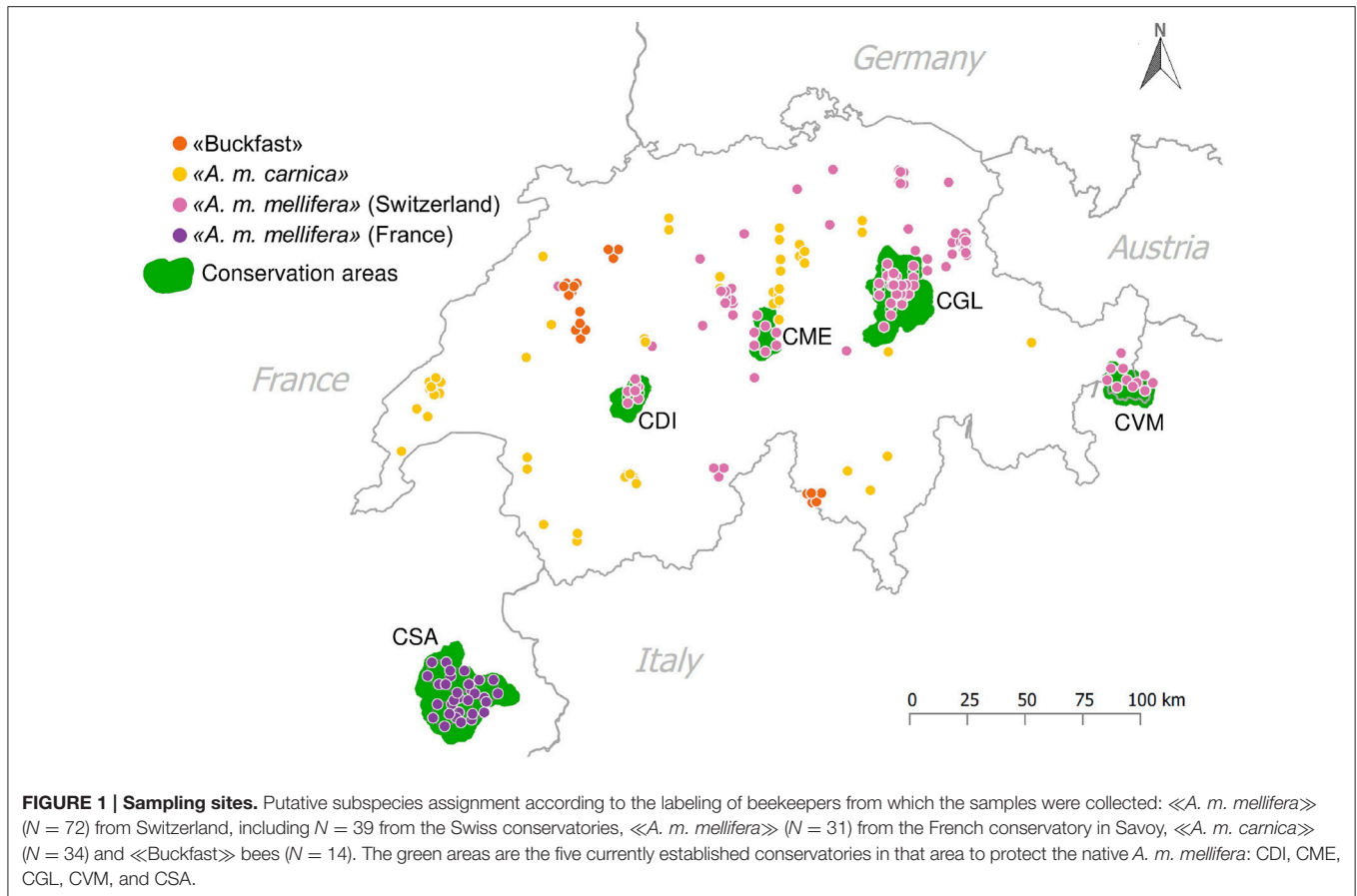
Complementary *A. m. mellifera* were sampled from a conservation area in the Savoie region in the French Alps (CSA: $N = 31$). These samples have been sequenced as part of a larger project, SeqApiPop, to characterize French honey bee populations. DNA extraction, library preparation and sequencing of these samples were performed as described in Wragg et al. (2016).

Mapping, Variant Calling, and Quality Control

Sequencing reads were mapped to the reference genome (Amel4.5) using *bwa mem* 0.7.10 (Li and Durbin, 2009) and duplicates marked using PICARD 1.80 (<http://picard.sourceforge.net>). Reads around indels were realigned with *RealignerTargetCreator* and *IndelRealigner* from the Genome Analysis Toolkit 3.3.0 (GATK) (McKenna et al., 2010; Van der Auwera et al., 2013). The base quality scores were recalibrated with GATK's *BaseRecalibrator*, using SNPs called with GATK's *UnifiedGenotyper* as covariates creating binary alignment/map (BAM) files for each sample ($N = 151$).

SNP calling was performed using a two-step process as described in Wragg et al. (2016). In brief, SNP variants were first identified in the Swiss bee sequence data ($N = 120$) applying three different variant calling tools: GATK's *UnifiedGenotyper* (Van der Auwera et al., 2013), SAMtools' *mpileup* 1.1 (Li et al., 2009) and PLATYPUS 0.8.1 (Rimmer et al., 2014). After variant calling SNPs were filtered according to base quality (BQ) score ≥ 20 and mapping quality (MQ) ≥ 30 . Calls from *UnifiedGenotyper* were additionally filtered for maximum number of alternate alleles = 2, genotype quality (GQ) ≥ 30 , quality by depth (QD) ≥ 2 , and Fisher strand (FS) ≤ 60 . After quality control, identified variants were combined using BAYSIC (Cantarel et al., 2014) which performs Bayesian latent class analysis to estimate false positive and false negative error rates. The resulting single-sample variant calling files (VCFs) were then merged together using bcftools (Li, 2011) and filtered on depth of coverage (DP) to generate a set of master sites mapped to chromosomes 1 to 16 with $9 \leq DP \leq 3 \times \text{mean DP}$. All individuals were re-genotyped with *UnifiedGenotyper* (BQ ≥ 20) at these master sites resulting in a multi-sample VCF comprising all samples.

Finally, variants were filtered using PLINK 1.9 (Chang et al., 2015) to exclude SNPs with minor allele frequency (MAF) < 0.01 and genotyping call rate < 0.9 , resulting in 3.375 M SNPs for subsequent analysis. Missing genotypes were imputed with ShapeIT v2 (O'Connell et al., 2014). DP was calculated with GATK's *DepthOfCoverage* tool, mapping and alignment metrics with PICARD's *CollectAlignmentSummaryMetrics* and



SAMtools' *flagstat*, and variant calling statistics with VCFtools *vcf-stats* (Danecek et al., 2011).

Population Structure Analyses

Principal Component Analysis (PCA)

We performed principal component analysis (PCA) to assess the population structure of the sampled honey bees. PCA is a classical non-parametric linear reduction technique used to reveal population structure by arranging all principal components (PCs) according to the explained variance without resorting to a model (Menozzi et al., 1978; Price et al., 2006; Gao and Starmer, 2008). Here, we applied PCA on a genetic relationship matrix ($n \times n$) with pairwise identities by state (IBS) between all individuals ($N = 151$) as provided by PLINK 1.9 (Chang et al., 2015). To infer the number of significant PCs and to determine significant differences between subpopulations, we used Horn's parallel analysis (PA; Horn, 1965; Dinno, 2009) as implemented in the R package *paran* (Dinno, 2012). After initial assessment of the population structure with PCA, we further explored the hierarchical structure using a network-based clustering approach and investigated individual admixture proportions using a model-based approach.

Hierarchical Population Structure

To determine the uppermost hierarchical population structure, we applied an unsupervised network-based clustering algorithm

called super paramagnetic clustering (SPC, Blatt et al., 1996; Tetko et al., 2005). The input to SPC is a dissimilarity matrix D ($n \times n$) with pairwise genetic distances between all individuals calculated using allele sharing distance (ASD; one minus IBS). Given D , each individual gets associated with a Pott spin variable (q). Once q have been assigned, the clustering is performed along a range of temperature (ΔT) limiting the interactions to a given number of k nearest neighbors (k -NN). We applied the algorithm using the following settings: k -NN = 15, $q = 20$ and (ΔT) = 0.01. For the visualization of hierarchical population structure and cluster solution, we used the R software package *phytools* (Revell, 2012).

Admixture

To estimate the admixture proportion of each individual, we performed model-based cluster analyses as implemented in the program ADMIXTURE (Alexander et al., 2009). The program was run unsupervised with 10,000 iterations and a pre-specified number of clusters K ($K = 1-6$). Convergence between independent runs at the same K was monitored by comparing the resulting log-likelihood scores (LLS) following 10,000 iterations, and was inferred from stabilized LLS with less than 1 LL unit of variation between runs. Cross validation (CV) error estimation for each cluster was performed with ADMIXTURE (Alexander et al., 2009) and used to determine the optimal number of K clusters.

To investigate the differences in introgression from introduced honey bees into the native *A. m. mellifera* gene pool, we compared admixture proportions from individuals originating from different sampling locations. For that reason, we calculated median and interquartile range (IQR) of the admixture proportions calculated at $K = 2$ from the samples in each of the conservation areas (CDI, CGL, CME, CVM, and CSA), as well as for the *A. m. mellifera* samples originating from unprotected regions in Switzerland (MEL). To evaluate the differences among these groups, a Kruskal-Wallis (Kruskal and Wallis, 1952) and *post-hoc* pairwise comparisons using Dunn's test (Dunn, 1964) with Holm correction (Holm, 1979) were conducted.

High-Resolution Population Networks

Model-based admixture analyses and network-based clustering were combined into high-resolution population networks in order to illustrate individual relationships and fine-scale population structure. We investigated high-resolution population networks using the open graph visualization platform Cytoscape (Shannon et al., 2003) and the plugin MultiColoredNodes (Warsow et al., 2010). In the final network visualization, the uppermost hierarchical population structure as inferred from SPC is presented in terms of node size, number of edges between nodes and width of edges. Here, we have associated the node size of each honey bee with the number of k -nearest neighbors (k -NN; number of edges), while the color of each honey bee represents the pre-determined level of admixture at $K = 2$ and $K = 4$, respectively. In order to express the strength of relationship between two individuals, the line width of an edge is proportional to the genetic relatedness between them (IBS). This approach is described in Neuditschko et al. (2012) and a recent implementation of this workflow is now available as R package (Steinig et al., 2015) posted at <https://github.com/esteinig/netview>.

In network theory, so-called communities, which are more densely and strongly connected within a group than outside a group, can be detected. Based on the high-resolution population network at $K = 4$ (see Result section), we selected a subset (communities) of core bees (gray dashed circles in **Figure 4B**) for each subpopulation which were clustering together and showed no or low levels of admixture (<0.1 ; except for Buckfast which was a more heterogeneous group). This subset consisted of a total of 95 core bees representing the four sampled subpopulations; 13 Buckfast bees, 26 *A. m. carnica*, 39 *A. m. mellifera* from Switzerland and 17 *A. m. mellifera* from France. These were used in subsequent population genetic analyses. The 56 remaining bees, which clustered outside the four identified communities, were designated to the subsample of test bees ($N = 56$) (Supplementary Table 1).

Population Differentiation and Genetic Diversity

To investigate average genome-wide divergence between the above defined subpopulations, we estimated mean pairwise population differentiation F_{ST} (Weir and Cockerham, 1984) in window sizes of 5 kb and 1 kb overlap using VCFtools (Danecek et al., 2011). We further estimated F_{ST} between native honey bees (*A. m. mellifera* from Switzerland and France) from the

evolutionary M-lineage and introduced honey bees (*A. m. carnica* and Buckfast) with mainly C-lineage ancestry. In addition, we estimated nucleotide diversity π in each subpopulation as defined by the average pairwise sequence difference per nucleotide site (Nei, 1982). We used VCFtools (Danecek et al., 2011) to calculate π in window sizes of 5 kb with 1 kb increment and estimated confidence intervals of the mean. It was not possible to calculate π for core bees originating from each of the Swiss conservation areas separately due to the limited sample size (<5) in CME, CDI, and CVM, thus π could only be calculated for CGL ($N = 15$).

Informative SNP Panels

SNP Thinning and Selection of Informative SNP Panels

The systemic homozygosity in haploid sequence data results in strong linkage disequilibrium (LD) between SNPs (see Supplementary Figure 1), and consequently a large number of uninformative or "redundant" SNPs (Weale et al., 2003; Nicolas et al., 2006; Paschou et al., 2008). To reduce SNP density and redundancy without adversely affecting LD-associated fine-structure, the dataset was randomly down-sampled to 10,000 SNPs using PLINK 1.9 (Chang et al., 2015). To ensure that there was no loss of information after the stringent thinning step, we computed and compared ASD-based distance matrices and PCA results between the entire (3.375 M SNPs) and thinned (10 K SNPs) datasets. First, to verify whether the genetic relationships between individuals are reflected in the thinned dataset, we tested the concordance between the ASD distance matrix with all bees ($N = 151$) inferred from the whole-genome (3.375 M SNPs) and the pairwise distances inferred from the thinned (10 K SNPs) dataset using Mantel R (Mantel, 1967). Mantel R is a permutation test to estimate the correlation between two matrices and was calculated using the package *vegan* in R (Oksanen et al., 2016). Second, to visualize whether the thinned dataset is able to capture the population structure revealed by all SNPs, we computed PCA for each dataset and plotted the core bees ($N = 95$) on the first and second PCs.

To distinguish introduced from native honey bees for conservation or breeding purposes, ancestry informative SNPs for subspecies discrimination were identified. Out of the thinned dataset, we generated panels with different number of SNPs (1000, 500, 100, and 50 SNPs) employing three different selection methods:

- (1) F_{ST} (Weir and Cockerham, 1984) between native and introduced honey bees. F_{ST} per site was calculated between core bees of *A. m. mellifera* from Switzerland and France ($N = 56$) and core bees of *A. m. carnica* and Buckfast bees ($N = 39$) using VCFtools (Danecek et al., 2011). Four SNP panels with the highest F_{ST} per SNP were defined as: $F_{ST-1000}$, F_{ST-500} , F_{ST-100} and F_{ST-50} . In addition, we selected a fifth panel correcting for any remaining redundancy in the SNP informativeness by computing a sorted QR-decomposition to effectively remove correlated markers (Paschou et al., 2008). The input for QR-decomposition is a genotype matrix with dimensions $n \times m$ (samples \times SNPs), where each SNP is encoded with either -1 for homozygous reference or 1 for homozygous alternate allele. Applying this approach

with a custom script in GNU octave (Eaton et al., 2014), we extracted the most uncorrelated SNPs out of the F_{ST} -100 panel resulting in the fifth SNP panel: *UncorrF_{ST}-50*.

- (2) PCA-derived, whereby a subset PCA-correlated SNPs were selected to reproduce the structure found by PCA without use of prior ancestry information (Paschou et al., 2007). This approach measures the correlation between a SNP and the significant principal components giving each SNP a PCA-score (Paschou et al., 2007). The input is a genotype matrix (samples \times SNPs) encoded as above (1, -1) and PCA-scores were calculated using a custom script in R. Finally, we defined four informative SNP panels with the highest PCA-scores: *PCA-1000*, *PCA-500*, *PCA-100*, and *PCA-50*. Additionally, we generated a fifth panel applying the same approach as for the *UncorrF_{ST}-50* (see above). We thus selected the 50 most uncorrelated out of the *PCA-100* SNPs resulting in the panel: *UncorrPCA-50*.
- (3) Randomly chosen SNPs, selected using the *sample*-function in R to generate panels with 1000, 500, 100, and 50 SNPs, respectively. Since the variation when using randomly selected SNPs is expected to be high, we run the *sample*-function five times each to produce a total of 20 SNP panels: 5x *Random-1000*, 5x *Random-500*, 5x *Random-100*, and 5x *Random-50*.

After generating the SNP panels, we tested their accuracy in estimating admixture proportions and genetic relationships between individuals by reference the earlier results from the complete SNP dataset. The random panel replicates were tested independently and the averaged statistic and variation are reported.

Performance of Informative SNP Panels

To compare the performance of the SNP panels on the accuracy to estimate admixture proportions, we ran ADMIXTURE (Alexander et al., 2009) at $K = 2$ using all bees ($N = 151$) and each of the selected SNP panels. The admixture proportions of the test bees ($N = 56$), which were not used for selection of the ancestry informative SNPs, were retrieved to test the accuracy of the SNP panels to predict admixture proportions. We therefore estimated the correlation coefficient r (Pearson, 1895) between the admixture proportions calculated based on all 3.375 M SNPs and the admixture proportions estimated with each of the selected SNP panels. Moreover, mean standard error (SE) of the inferred admixture proportions of all bees was calculated for each of the SNP panel, in order to examine whether lower numbers of SNPs increase SE.

To investigate the precision of the selected panels to predict the genetic relatedness between individuals, we computed pairwise distances (ASD) between all samples ($N = 151$) with each of the selected SNP panels. To estimate the concordance between the resulting distance matrices and the ASD matrix inferred from the entire data set (3.375 M SNPs), we performed pairwise Mantel tests (Mantel, 1967).

Finally, in order to visualize the population differentiation, we also computed PCA with the best performing panel and plotted the first two PCs. All statistics, if not otherwise stated, were calculated and plotted in R (R Core Team, 2016).

RESULTS

Mapping and Variant Calling

On average 19,549,894 paired-end reads were generated per bee of which 96.3% mapped in pairs to the reference genome Amel4.5 (Elsik et al., 2014). Approximately 2.2% of the mapped reads were marked as duplicates that aligned with the identical start and end positions onto the reference genome. Sequencing depth per sample ranged from 3.3 to 20.7X with mean 9.9X resulting in 97.2% of the genome being covered on average. Individual mapping and alignment statistics are presented in Supplementary Table 2.

Individual variant calling statistics for each drone genome in the Swiss dataset, comparing the three different applied variant calling tools, can be found in Supplementary Table 3. Once filtered on depth of coverage, the master variant file identified by combining single sample VCF files resulted in 4.986 M raw SNPs, which were used to genotype both the Swiss and French datasets. Post quality control filtering, 3,374,686 high-quality genome-wide SNPs were retained for analysis.

Population Structure Analyses

Principal Component Analysis (PCA)

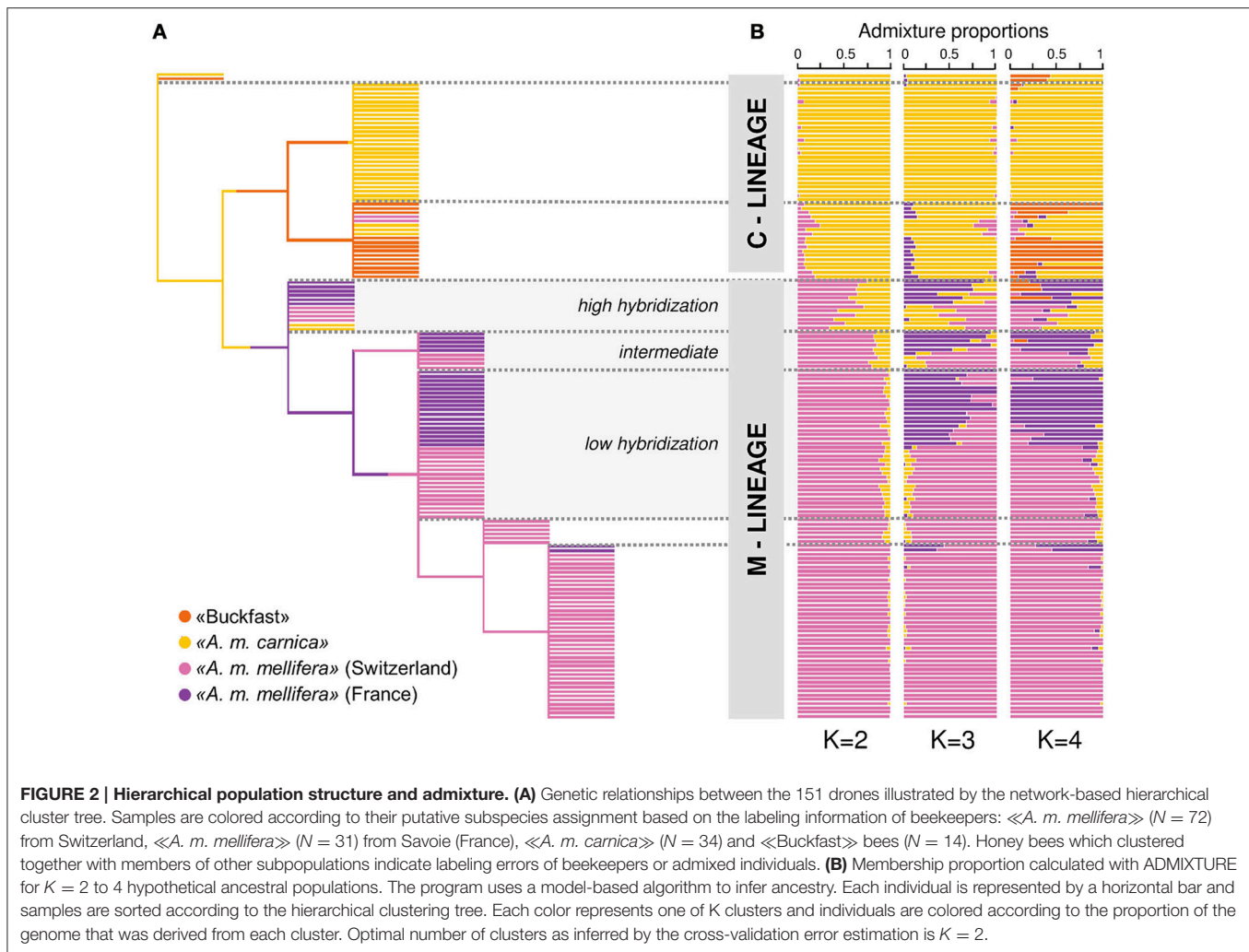
The result of PCA shows that the first PC clearly separates the samples into two major clusters according to the two evolutionary lineages M and C, while further substructures within these lineages are revealed on the second PC (Supplementary Figure 2). The general diversity pattern is thus characterized by four populations: *A. m. carnica* and the diverse Buckfast group within the C-lineage cluster and two subpopulations of *A. m. mellifera* within the M-lineage cluster originating from Switzerland and France, respectively. Horn's PA resulted in the retention of one significant PC accounting for 95.1% of the variance. This demonstrates a significant separation of the samples according to the evolutionary lineages, while the substructures disclosed on the second PC are not significant.

Hierarchical Population Structure

The network-based cluster-tree recapitulates the findings of PCA by dividing the three honey bee subspecies (*A. m. mellifera*, *A. m. carnica*, and Buckfast) into two distinct population clusters, while two honey bees were assigned into an additional single cluster (Figure 2A). Moving up the cluster tree, *A. m. carnica* and Buckfast were assigned into respective population clusters and honey bees with mixed genetic origin were separated from the main *A. m. mellifera* population. At the additional levels of the tree, *A. m. mellifera* was further sub-structured, whereas the identified sub-structures do not reflect the different geographic origin of samples. On the top level of the tree, especially *A. m. mellifera* sampled from the four Swiss conservation areas were clustered together along with two samples from France. The final topology of the network-based cluster tree shows that honey bees are characterized by a high level of hierarchical structure.

Admixture

Following the CV error estimation (Supplementary Figure 3), ADMIXTURE (Alexander et al., 2009) suggested an optimal number of clusters $K = 2$. Given $K = 2$, individuals are



separated into two major clusters which correspond to the two distant evolutionary lineages M and C (Figure 2B). The samples, which are sorted according to the hierarchical clustering tree, appear arranged in groups depending on their degree of admixture (Figure 2B). Hence, the admixture levels at $K = 2$ identified by ADMIXTURE (Alexander et al., 2009), which uses a model-based approach, reflect the hierarchical structure found by the network-based clustering tree (Figure 2A). At increasing values of K , further substructures become evident which are in concordance with the sampled subpopulations or their geographic origin. At $K = 3$, the M-lineage cluster is separated into *A. m. mellifera* bees with Swiss and French ancestry, respectively. Increasing K to 4, subdivides the C-lineage cluster into honey bees with *A. m. carnica* ancestry and Buckfast ancestry.

If samples are ordered according to sampling locations, admixed individuals are found in all of the five sampled conservation areas (Supplementary Figure 4). The lowest median admixture levels were found in the CGL conservatory (Mdn = 0.000, IQR = 0.000–0.007), followed by CME (Mdn = 0.059, IQR = 0.013–0.077), CVM (Mdn = 0.071, IQR = 0.032–0.314) and the French conservatory CSA (Mdn = 0.072, IQR = 0.034–0.178)

(Figure 3). The highest median admixture levels were found in CDI (Mdn = 0.073, IQR = 0.063–0.083). Excepting for a few highly admixed samples, *A. m. mellifera* originating from unprotected areas were generally little admixed (Mdn = 0.030, IQR = 0.014–0.091). The mean ranks of admixture proportions among these six sampling locations were significantly different (Kruskal-Wallis: $H = 22.95$, $df = 5$, $P = 0.0003$). Follow-up tests indicated a significant difference between CGL and CVM (Dunn's $z = -3.59$, $p = 0.002$), CGL and CSA (Dunn's $z = -4.41$, $p < 0.001$), and CGL and the samples from unprotected areas (MEL) (Dunn's $z = -3.02$, $p = 0.017$) (Figure 3).

High-Resolution Population Networks

The high resolution population networks illustrate individual relationships and fine-scale population structure (Figure 4). The network at optimal $K = 2$ reveals a gradual arrangement of individuals according to their degree of M- and C-lineage ancestry, respectively (Figure 4A). Yet, substructuring is clearly evident when nodes are colored according to admixture proportions at $K = 4$ (Figure 4B). Here, the Buckfast cluster separates from the *A. m. carnica* cluster and, in addition, within the M-lineage cluster there is

substructuring of the *A. m. mellifera* bees originating from France (Figure 4B).

The densely packed *A. m. mellifera* cluster from Switzerland, as well as the *A. m. carnica* cluster, reveal a high genetic relatedness of individuals within these populations. This is also reflected in the greater edge widths (=pairwise IBS) and node

sizes (=number of nearest neighbors) within these clusters. Whereas, highly admixed individuals are clustered together based on their degree of admixture and not because of higher genetic relationship, as shown by the thinner edges between nodes. The high resolution visualization allows further to identify outliers, one in the Swiss *A. m. mellifera* cluster and two in the French *A. m. mellifera* cluster, which are connected to one other individual only. These honey bees are not closely related to the rest of the cluster and represent rare genetic variation.

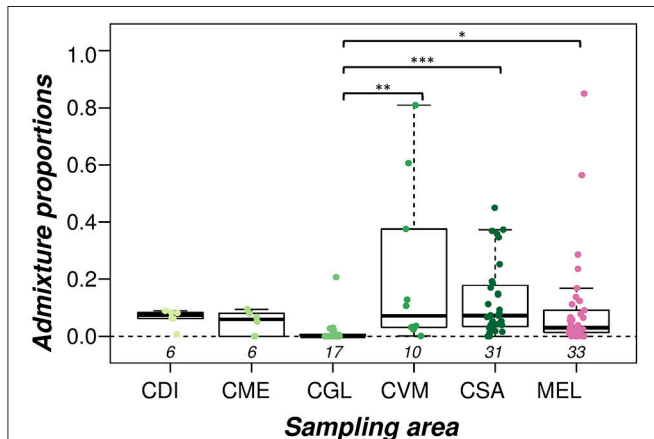


FIGURE 3 | Admixture proportions of *A. m. mellifera* sampled outside (MEL) and within each of the conservation areas (CDI, CGL, CME, CVM, and CSA). The box denotes the upper and lower quartiles, and the median is represented by a solid black line within the box. There is a significant difference between the mean ranks of the admixture proportions among the different sampling areas (Kruskal-Wallis: $H = 22.95$, $df = 5$, $P = 0.0003$). The conservation area CGL has significantly lower admixture levels than the CVM and CSA conservatories, as well as compared to *A. m. mellifera* sampled outside conservation areas (MEL) (pairwise multiple comparisons using Dunn's test and Holm correction, significance levels marked as: $p < 0.05$, $p > 0.01$ **, $p > 0.001$ ***). Numbers below boxplots designate sample size in each group.

Population Differentiation and Genetic Diversity

Overall, average genome-wide population differentiation between each of the core bee subpopulations (as defined in Material and Methods; Supplementary Table 1) is characterized by high divergence between and low divergence within the evolutionary lineages M and C. The lowest level of population differentiation was found between the Swiss and French *A. m. mellifera* ($F_{ST} = 0.023$), while the highest difference was observed between the Swiss *A. m. mellifera* and the *A. m. carnica* ($F_{ST} = 0.359$) (Table 1). Introduced honey bees (*A. m. carnica* and Buckfast, mainly from the C-lineage) are strongly differentiated from the native *A. m. mellifera* populations (M-lineage) ($F_{ST} = 0.317$).

Genetic diversity was highest in the Buckfast bee population [$\pi = 0.00392$, 95% CI (0.00391, 0.00393)], followed by the *A. m. mellifera* populations from the French conservatory [$\pi = 0.00354$, 95% CI (0.00353, 0.00355)] and Switzerland [$\pi = 0.00331$, 95% CI (0.00330, 0.00332)]. The lowest level of genetic diversity was found in the *A. m. carnica* population [$\pi = 0.00309$, 95% CI (0.00308, 0.00310)]. Nucleotide diversity of the Swiss conservatory CGL [$\pi = 0.00319$, 95% CI (0.00318,

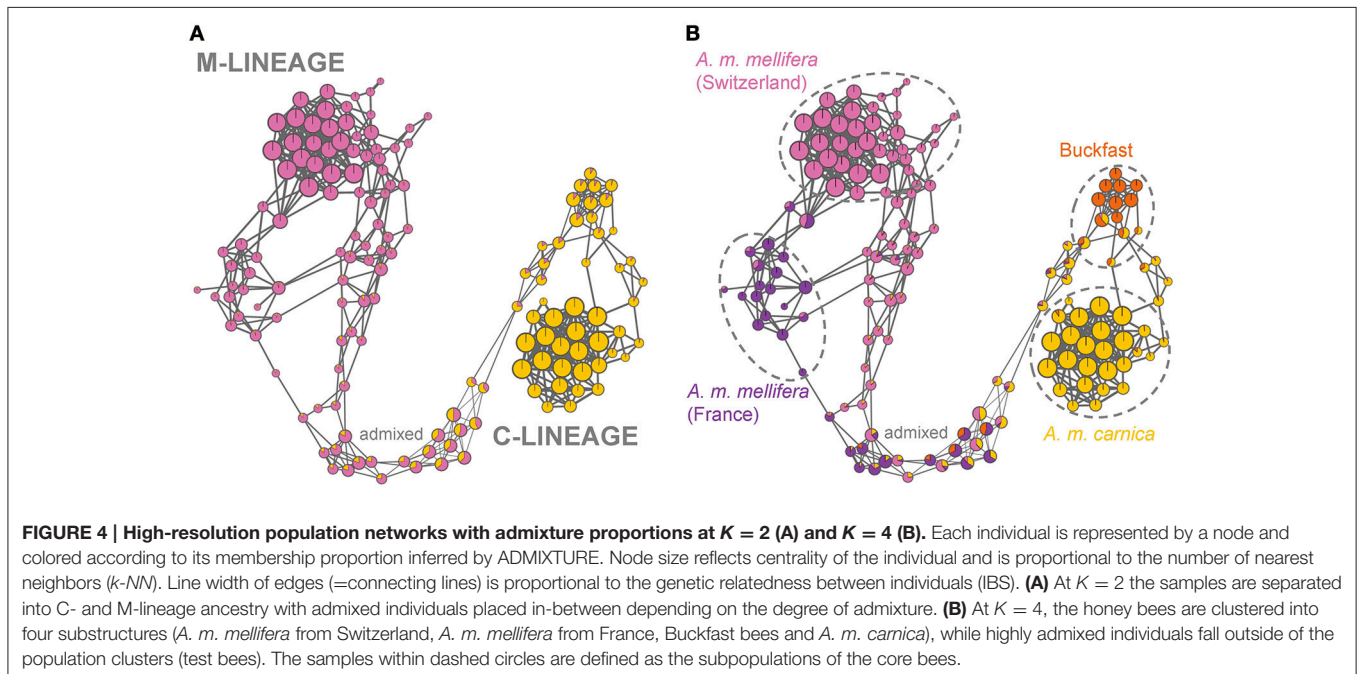


FIGURE 4 | High-resolution population networks with admixture proportions at $K = 2$ (A) and $K = 4$ (B). Each individual is represented by a node and colored according to its membership proportion inferred by ADMIXTURE. Node size reflects centrality of the individual and is proportional to the number of nearest neighbors ($k-NN$). Line width of edges (=connecting lines) is proportional to the genetic relatedness between individuals (IBS). (A) At $K = 2$ the samples are separated into C- and M-lineage ancestry with admixed individuals placed in-between depending on the degree of admixture. (B) At $K = 4$, the honey bees are clustered into four substructures (*A. m. mellifera* from Switzerland, *A. m. mellifera* from France, Buckfast bees and *A. m. carnica*), while highly admixed individuals fall outside of the population clusters (test bees). The samples within dashed circles are defined as the subpopulations of the core bees.

TABLE 1 | Mean pairwise population differentiation (F_{ST}) based on 3.375 M SNPs.

		Lineage		
		M		C
Population		<i>A. m. mellifera</i> (S)	<i>A. m. mellifera</i> (F)	<i>A. m. carnica</i>
Lineage	M	<i>A. m. mellifera</i> (F)	0.023	
	C	<i>A. m. carnica</i>	0.359	0.350
	Buckfast		0.330	0.297
				0.041

Average genome-wide F_{ST} values were calculated with VCFtools in window sizes of 5 kb and 1 kb overlap between each of the four honey bee subpopulations of core bees; Buckfast ($N = 13$), *A. m. carnica* ($N = 26$), *A. m. mellifera* from Switzerland ($N = 39$) and from France ($N = 17$).

0.00320)] was significantly lower compared to the French conservation area CSA (Welch's $t = -53.6$, $df = 398900$, $p < 0.001$).

Informative SNP Panels

SNP Thinning

The pairwise genetic distance matrices calculated using the total (3.375 M SNPs) and the thinned (10 K SNPs) datasets are highly concordant [Mantel $R = 0.999$, 95% CI (0.998, 0.999), $p < 0.001$]. Moreover, when visualized the PCA scatter plot generated with the thinned dataset (10 K SNPs) reflects the same pattern found using all SNPs, yet with a less clear distinction between Swiss and French *A. m. mellifera* (Figures 5A,B). Hence, the population structure and genetic relationships observed using all SNPs is conserved well with the 10 K SNPs dataset, with only minimal loss of information despite the stringent thinning step applied and could thus be used to select ancestry informative SNPs.

Performance of Informative SNP Panels

Irrespective of the selection method, there was a strong positive correlation between the admixture proportions calculated using all SNPs (3.375 M) and the admixture proportions calculated with each of the reduced SNP panels (Pearson's $r > 0.90$, $p > 0.001$ for all panels; Figure 6A). The correlation decreases with decreasing number of SNPs and there is no difference between the three tested selection methods (F_{ST} , PCA and Random) (Figure 6A; left panel). However, there is a significant difference when the $UncorrF_{ST-50}$ panel, which is additionally corrected for any remaining redundancy in SNP informativeness, is compared to the other SNP panels with only 50 SNPs. The $UncorrF_{ST-50}$ panel significantly better predicts admixture proportions than the $Uncorr50-PCA$ panel [Fisher's $z = 2.96$, $p = 0.003$; 95% CI for the difference between correlations (0.02–0.10)], and better than 50 randomly selected SNPs [Fisher's $z = 2.76$, $p = 0.006$; 95% CI for the difference between correlations (0.02–0.09)]. Moreover, the standard errors of the mean admixture proportions increase with decreasing number of SNPs and with 50 SNPs only, the $UncorrF_{ST-50}$ had significantly lower mean SE [$\bar{x} = 0.043$, 95% CI (0.046, 0.039)] than the other selection methods (Supplementary Table 5).

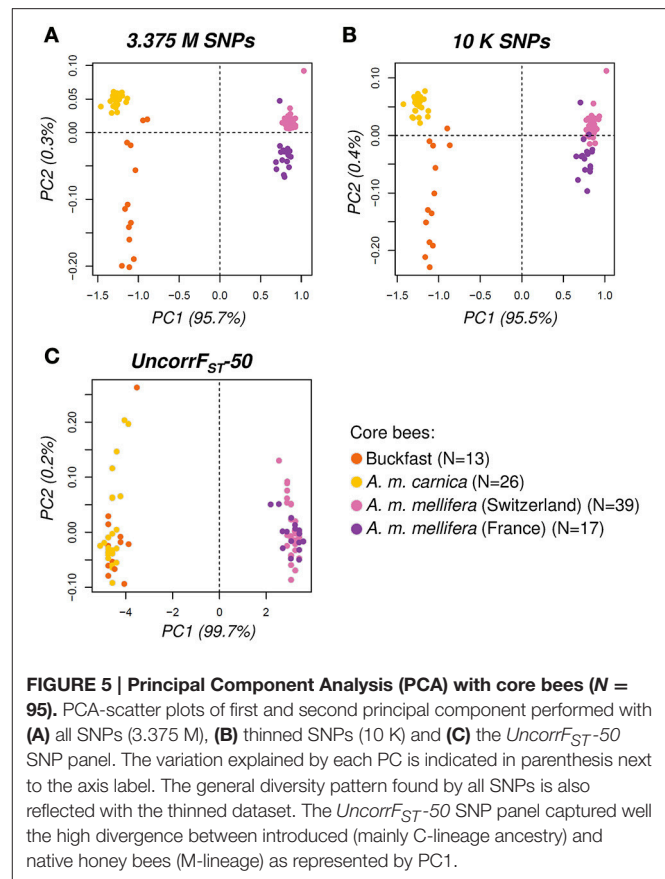


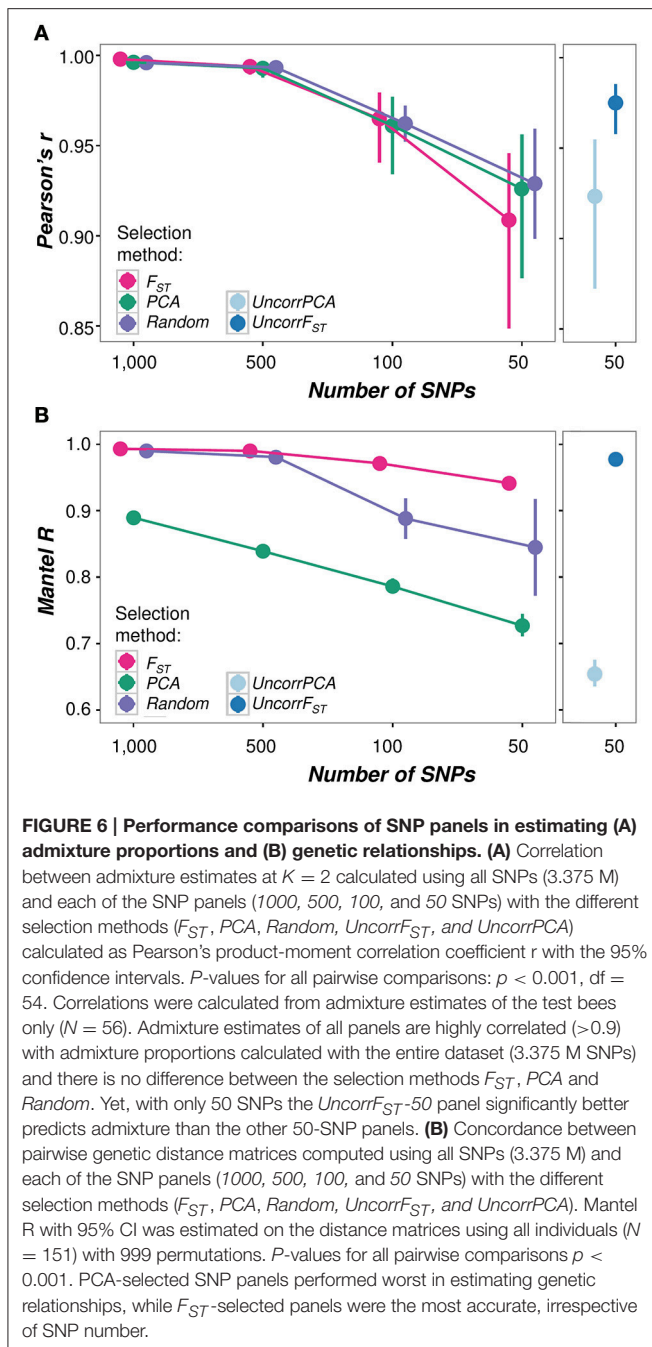
FIGURE 5 | Principal Component Analysis (PCA) with core bees ($N = 95$). PCA-scatter plots of first and second principal component performed with (A) all SNPs (3.375 M), (B) thinned SNPs (10 K) and (C) the $UncorrF_{ST-50}$ SNP panel. The variation explained by each PC is indicated in parenthesis next to the axis label. The general diversity pattern found by all SNPs is also reflected with the thinned dataset. The $UncorrF_{ST-50}$ SNP panel captured well the high divergence between introduced (mainly C-lineage ancestry) and native honey bees (M-lineage) as represented by PC1.

SNP panels generated based on the PCA informativeness consistently performed poorest when assessing their accuracy for inferring genetic relationships between individuals, while SNP panels generated based on F_{ST} performed best, as indicated by the non-overlapping confidence intervals in Figure 6B. Considering only 50-SNPs, the $UncorrF_{ST-50}$ significantly better reflects the genetic relationships between individuals than the 50-SNP panels based on the other selection methods.

In conclusion, with only 50 SNPs the $UncorrF_{ST-50}$ panel clearly outperformed the other selection methods (SNP positions in Supplementary Table 4). Admixture proportions computed with the $UncorrF_{ST-50}$ panel were very highly correlated with admixture proportions using the whole-genome (3.375 M SNPs) dataset [Pearson's $r = 0.975$, 95% CI (0.958, 0.985), $p < 0.001$] while accounting for the lowest error [$SE = 0.043$, 95% CI (0.039, 0.046)]. Additionally, the genetic relationships inferred from the $UncorrF_{ST-50}$ panel were also highly correlated with all 3.375 M SNPs [Mantel $R = 0.977$, 95% CI (0.976, 0.980), $p < 0.001$]. The PCA scatter plot (Figure 5C) visually clearly highlights the ability of the $UncorrF_{ST-50}$ SNP panel to reveal the significant difference and to separate introduced from native honey bees, as shown by the first PC explaining 99.7% of the variance.

DISCUSSION

Network-based clustering was used for the first time in honey bees enabling effective identification of fine-scale population



structure and distinct separation of introduced and native honey bees corresponding to highly divergent lineages. Moreover, substructures within *A. m. mellifera* were identified according to origin (Switzerland or France). Finally, despite current ongoing conservation efforts, admixed individuals were still found in all conservation areas thereby calling for improved management practices. Our results show that 50 highly informative SNPs could be a cost-effective tool to enhance conservation efforts as they are sufficient to accurately detect C-lineage introgression in the sampled native honey bee populations.

Population Structure

To analyze population structure, we employed a set of different cluster approaches which all revealed a significant and clear separation between the well-known evolutionary M- and C-lineage (Ruttner, 1988; Garnery et al., 1992). But, as indicated by the final topology of the network-based clustering tree (Figure 2A), honey bees are further characterized by a high level of hierarchical structure. By integrating the model-based admixture results in the final network visualization, we can thus see that the admixture proportions are related to the genetic relationship between individuals. It is well known, but not often discussed, that model-based approaches, such as those implemented in ADMIXTURE, are highly dependent on the reference sampling populations and rely on prior assumptions (Pritchard et al., 2000; Greenbaum et al., 2016). For instance, highly related individuals can form an artificial substructure showing no or very little admixture, and as a consequence more distantly related individuals show increased levels of admixture (Pritchard et al., 2000). This emphasizes the importance to be cautious when interpreting admixture levels and when possible to choose diverse and unrelated individuals. In our case, due to the sampling bias of many more Swiss than French *A. m. mellifera* samples, admixture levels in the latter could thus be slightly overestimated. Ideally, to overcome the sampling bias, an optimized approach would be to combine network clustering with model-based analyses and classical PCA, as these complement each other and thus give a more robust and detailed picture (Neuditschko et al., 2012; Greenbaum et al., 2016). This is the advantage of our combined network visualization; it illustrates both the genetic relationships between individuals and admixture levels, which is crucial for the interpretation of the observed population structures (Neuditschko et al., 2012). Therefore, the network clustering is highly suitable to identify community structures corresponding to the four subpopulations in this dataset (*A. m. carnica*, Buckfast, *A. m. mellifera* from Switzerland and France). Furthermore, admixed individuals can be easily spotted.

The substructure between Swiss and French *A. m. mellifera* is surprising, given the large mating distances of honey bee drones from their colony of origin (Neumann et al., 1999c). Here, we found already some population differentiation on a short distance of ~40 km between the conservatory in the French Alps and Swiss *A. m. mellifera*. The reasons for this finding can be manifold including genetic drift, differential beekeeping practices or even local adaptations. Isolation-by-distance could be explained with the Alps acting as natural barriers against gene flow between North (Switzerland) and South (France). However, within Switzerland no such effect could be detected even though massive mountain ranges separate native bees in different valleys. As honey bee queens are bought and sold throughout the country one could expect less or no substructures within Switzerland due to human-mediated gene-flow. Alternatively, there might be locally adapted ecotypes within *A. m. mellifera*. Given the large native range of *A. m. mellifera* (from France over Central Europe, the British Isles and Southern Scandinavia to the Ural Mountains, Ruttner, 1988), it does not seem surprising that there are substructures or ecotypes within this subspecies. For

instance, ecotypes of *A. m. mellifera* with particular brood cycles adapted to the local flora have already been described in France (Louveaux et al., 1966; Strange et al., 2007, 2008). More importantly, with regard to honey bee health, locally adapted honey bee populations may have evolved their own specific resistance mechanisms matching the prevailing pathosphere they are located in (genotype-environment interactions) (Meixner et al., 2015). In order to protect and promote native honey bees it is therefore essential to identify substructures and locally adapted ecotypes.

Genetic Diversity

All identified populations, including both native French and Swiss honey bees exhibit high genetic variation, which is comparable to other tested populations from C- and M-lineage or mixed background, respectively (Wallberg et al., 2014; Wragg et al., 2016). This genetic diversity is important to preserve as it harbors the adaptive potential for future needs (Frankham et al., 2002) and enhances productivity and fitness (Mattila and Seeley, 2007). On the other hand, genetic admixture through the importation and hybridization with distant subspecies can disrupt the locally adapted genetic variation (De La Rúa et al., 2009). The lowest genetic diversity was found in the *A. m. carnica* population, which was introduced into Switzerland in the 1960's, becoming very popular for apiculture and as a consequence was increasingly managed. The higher artificial selection pressure on this subspecies could therefore explain the lower genetic diversity. Similarly, populations of the C-lineage from other published studies (e.g., Wallberg et al., 2014; Wragg et al., 2016) exhibited lower genetic diversity and were sampled from selective breeding populations. An alternative explanation, however, could be a lower effective population size through a founder effect when introducing *A. m. carnica* to Switzerland in the first place. The highest genetic diversity was found in the hybrid Buckfast, which is in line with previous work that showed that high genetic diversity can be promoted through admixture by human management in mixed domestic bee populations (Harpur et al., 2012; Wallberg et al., 2014).

Admixture in the Conservation Areas

Despite the high genetic diversity found in native populations, we also identified some highly admixed individuals in the conservation areas, which is a concern and indicates that the management practices may need some adjustment. A previous analysis of 12 microsatellites in the Swiss *A. m. mellifera* breeding population sampled in 2003 showed similar levels of admixture (Soland-Reckeweg et al., 2009). More recently, in 2013, an analysis of 1381 SNPs in *A. m. mellifera* populations sampled throughout Europe also found admixed individuals in Switzerland (Pinto et al., 2014). While it is not possible to directly compare these studies because of the different sampling strategies and methodologies employed, all studies including the present one show that despite ongoing conservation efforts in Switzerland considerable C-lineage introgression in the *A. m. mellifera* population remains. Therefore, protection of the native population should be reinforced.

Yet, there are some differences in admixture levels between the five sampled conservation areas, which could be attributed to varying management practices. Alternatively, such differences might be linked to the time since the establishment of the conservatories. The Glarus conservatory (CGL) is the oldest (established in 1977) and largest conservation area in Switzerland, and shows the lowest level of C-lineage introgression. On the other hand, the Val Mustair conservatory (CVM), a remote region in southeastern Switzerland with a long native bee keeping tradition, but officially established in 2006 only, suffers from increased admixture. After inquiring with the management of this particular conservatory the increased level of admixture is the result of an *A. m. carnica*-beekeeper, who recently moved to the area. Thus, our data demonstrates that the genetic consequences are immediately measurable. Moreover, there is a need for an increased legal protection of conservation areas, as evidenced by the drastic effects of this single migratory beekeeper on conservation efforts. The French conservatory in Savoie (CSA) also displays admixed individuals. In this breeding program identification has been mainly based on morphometrics, which can have less resolution than genetic markers (Francis et al., 2014). In addition, France has a long history of foreign queen importation with many large-scale commercial apiarists keeping C-lineage bees (Franck et al., 1998; Garnery et al., 1998; Wragg et al., 2016). This increases the chance of introgression in the native gene pool.

Surprisingly, we found little admixture in native bees sampled from outside conservation areas. Our sampling scheme did not include randomly sampled bees from all of Switzerland, but depended on the willingness of beekeepers to participate. Thus, we would expect that there might be different levels of admixture under a totally random and unbiased sampling scheme. Yet, our results indicate that the keeping of pure native bees even outside a protected area is possible. To do so, motivated beekeepers involved in the maintenance and conservation of *A. m. mellifera* bring their virgins queens to controlled mating stations or buy queens from certified breeders. Our results suggest that this approach seems successful in Switzerland.

In contrast, maintaining freely-mating populations with minimal risk of introgression will require considerable monitoring efforts given the relatively small size of Swiss conservation areas. Consequently, in the longer term there is a need for the implementation of an enhanced international conservation strategy to preserve the native subspecies *A. m. mellifera* in apiculture. To this end, it is important to consistently and repeatedly test all colonies in the conservation areas for C-lineage introgression because of the natural movement of honey bees. Traditionally, morphometric analyses and more recently, microsatellite markers have been employed to detect and replace hybrid colonies (Soland-Reckeweg et al., 2009). An even more accurate tool would be a set of diagnostic SNPs to control mating stations and conservation areas as shown in this study (Hauser et al., 2011; Francis et al., 2014).

Informative SNP Panels

The high differentiation between introduced bees (*A. m. carnica* and Buckfast) belonging mainly to the evolutionary C-lineage

and the native *A. m. mellifera* from the M-lineage, allowed to straightforwardly identify ancestry informative or even fixed SNPs. This high differentiation between the M- and C-lineages is also the reason why even randomly selected SNPs performed relatively well compared to other selection methods. Yet, the results clearly show that as few as 50 most informative SNPs are sufficient to distinguish native from introduced honey bees. The *UncorrF_{ST}-50* panel outperformed the other 50-SNP panels in both the ability to predict admixture as well as genetic relationships, thereby suggesting their potential for a successful application in sustainable bee breeding programs. Such low density SNP panels have been developed successfully for breeding and breed assignment for a number of different livestock species (Boichard et al., 2012; Wellmann et al., 2013; Henshall et al., 2014). For honey bees, Muñoz et al. (2015) have previously generated sets of ancestry informative markers (AIMs) based on different selection methods to infer admixture between *A. m. mellifera* and C-lineage (*A. m. carnica* and *A. m. ligustica*). However, due to the hybrid origin of Buckfast bees, it might pose more difficulties to detect admixture with Buckfast than with pure C-lineage bees. Moreover, the full dataset of Muñoz et al. (2015) consisted of 1183 pre-selected SNPs from a previous study, while here we selected the most informative SNPs from whole-genome sequence information.

The application of only 50 SNPs might seem quite low for geneticists working with other livestock animals. However, given the fact that the beekeeping industry in Switzerland, and in most parts of Europe is based on leisure apiculturists (Chauzat et al., 2013), there is a general need for an economic approach to foster participation in conservation efforts. However, there is a trade-off between resolution and economics which poses a minor risk of failing to detect very low levels of admixture. A brief inquiry on current genotyping costs (August 2016) revealed the price per SNP as little as 0.50 €, which is expected to decrease even further thereby enabling the cost-effective testing of thousands of bees for a continental conservation strategy across Europe. Due to the low number of SNPs required for admixture analyses, it would also be possible to enhance stakeholder acceptance by including additional SNPs linked to traits of commercial interest, as is already implemented in custom SNP panels for other livestock species (Schwenger et al., 1993; Pannier et al., 2010). For honey bees, one such example could be to incorporate SNPs linked to resistance against *Varroa destructor* (Haddad et al., 2016; Spötter et al., 2016), given that this ectoparasitic mite is a significant contributor to global honey bee colony losses (Guzmán-Novoa et al., 2010; Dainat et al., 2012).

CONCLUSION

In this study, we demonstrated that the combination of model-based and network-based clustering allows us to identify high-resolution population structures between subspecies, which can be especially useful to identify locally adapted honey bee populations for conservation purposes and management

decisions optimizing genetic diversity. For honey bees high genetic diversity, as we found in all identified populations, is particularly important for colony health (Tapy, 2003) and entails the potential to adapt to new environmental conditions such as climate change or novel diseases.

As observed by this and other studies, hybrid colonies can be found throughout the native range of *A. m. mellifera* (Jensen et al., 2005a; Soland-Reckeweg et al., 2009; Pinto et al., 2014). It is clear that in such a diverse situation it is not possible to entirely prevent the natural movement of these hybrids and of foreign honey bees into conservatories. As a result, despite current ongoing conservation efforts, admixed individuals were still found in all conservation areas albeit with varying degrees of introgression thereby calling for improved management practices.

The use of ancestry informative SNPs for subspecies discrimination and estimation of admixture can thereby prove to be a very useful tool for enhancing conservation management efforts. We demonstrated that the selected ultra-low density SNP panel is accurate to estimate admixture and genetic relationships between the sampled honey bees. We therefore hope to contribute to the conservation efforts in order to ensure the valuable pollination services provided by our honey bees in the future.

DATA ACCESS

Swiss bee sequence data is deposited at the European Nucleotide Archive (ENA: <http://www.ebi.ac.uk/ena>) under study accession number: PRJEB16533. The sequence data of the bees from Savoie are available through the SeqApiPop programme on the Sequence Read Archive (SRA: <https://www.ncbi.nlm.nih.gov/sra>) with accession: SRP069814.

AUTHOR CONTRIBUTIONS

Conceived and designed the study: MP, LG, PN, MN. Collected data: MP, LG. Analysed and interpret the data: MP, DW, AV, PN, MN. Wrote and approved final manuscript: MP, DW, LG, AV, PN, MN.

FUNDING

This work was funded by the Swiss Federal Office for Agriculture FOAG and the Fondation Sur-la-Croix, Basel. Data for the Savoie samples was provided by the SeqApiPop programme, funded by the FranceAgriMer grant 14-21-AT. The GeT platform, Toulouse (France), is a partner of the National Infrastructure France Génomique, thanks to support by the Commissariat aux Grands Investissements (ANR-10-INBS-0009).

ACKNOWLEDGMENTS

Sequencing was performed at the GeT PlaGe platform in Toulouse, France. Bioinformatic analyses were performed

on UBELIX (<http://www.id.unibe.ch/hpc>), the HPC cluster at the University of Bern, Switzerland. We thank the beekeeping associations VSMB, SAR, SCIV, BIVS, VDRB, and CETA de Savoie and all beekeepers for providing the samples.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fevo.2016.00140/full#supplementary-material>

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Conflict of Interest Statement: 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.

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Plastic-Film Mulching for Enhanced Water-Use Efficiency and Economic Returns from Maize Fields in Semiarid China

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OPEN ACCESS

Edited by:

Evangelia G. Drakou,
University of Western Brittany, France

Reviewed by:

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and IPEE RAS, Russia
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 06 December 2016

Accepted: 23 March 2017

Published: 06 April 2017

Citation:

Zhang P, Wei T, Cai T, Ali S, Han Q,
Ren X and Jia Z (2017) Plastic-Film
Mulching for Enhanced Water-Use
Efficiency and Economic Returns from
Maize Fields in Semiarid China.
Front. Plant Sci. 8:512.
doi: 10.3389/fpls.2017.00512

Film mulch has gradually been popularized to increase water availability to crops for improving and stabilizing agricultural production in the semiarid areas of Northwest China. To find more sustainable and economic film mulch methods for alleviating drought stress in semiarid region, it is necessary to test optimum planting methods in same cultivation conditions. A field experiment was conducted during 2013 and 2014 to evaluate the effects of different plastic film mulch methods on soil water, soil temperature, water use efficiency (WUE), yield and revenue. The treatments included: (i) the control, conventional flat planting without plastic film mulch (CK); (ii) flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); (iii) furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); (iv) furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); and (v) furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat without plastic film-mulched space (60 cm wide). Topsoil temperature (5–25 cm) was significantly ($p < 0.05$) higher in field plots with plastic film mulch than the control (CK), and resulted in greater soil water storage (0–200 cm) up to 40 days after planting. Maize grain yield and WUE were significantly ($p < 0.05$) higher with the furrow planting methods (consecutive film-mulched ridges and alternating film-mulched ridges) than the check in both years. Maize yield was, on average, 29% ($p < 0.05$) greater and 28% ($p < 0.05$) greater with these furrow planting methods, while the average WUE increased by 22.8% ($p < 0.05$) with consecutive film-mulched ridges and 21.1% ($p < 0.05$) with alternating film-mulched ridges. The 2-year average net income increased by 1559, 528, and 350 Chinese Yuan (CNY) ha⁻¹ with the consecutive film-mulched ridges, furrow-flat planting and alternating film-mulched ridges, respectively, compared with the control (CK). We conclude that the consecutive film-mulched ridge method was the most productive and profitable for maize in this semi-arid area with limited and erratic precipitation.

Keywords: crop growth, film mulch, maize yield, rainfed area, soil temperature, soil water storage

INTRODUCTION

Dryland farming, which is practiced on about one-third of the arable land the Loess plateau, Northwest China, is constrained by the semiarid growing conditions (Li and Xiao, 1992). Precipitation during the growing season occurs mainly in the form of light rain showers and rainstorm, which contribute to soil erosion and water loss through runoff. The natural rainfall regime is not effective in supplying water at critical crop growth stages and recharging soil water reserves, resulting in frequent drought (Li et al., 2001). Spring maize (*Zea mays* L.) is one of the major crops in this region, accounting for 27.3% of the total agricultural area (Liu et al., 2010), but limited water availability (Du et al., 2010; Zhou et al., 2012) and erratic precipitation often lead to low maize yields and crop failure in some cases (Gan et al., 2013). As well, low soil temperature at the seedling stage can impede maize development and growth (Ramakrishna et al., 2006). Hence, the key to stabilizing and increasing maize yields in this region is to boost WUE from precipitation. This involves better methods of capturing, reducing evaporation and alleviating low soil temperatures in spring.

Plastic film mulch is widely used as a low-cost measure to improve water retention in the soil (Wang et al., 2009), increase soil temperature (Liu et al., 2010) and reduce soil evaporation (Li and Xiao, 1992). It provides economic benefits to the farmer because it promotes crop development, achieve an early harvest and increase maize yield, according to short- and long-term research (Liu et al., 2009; Steinmetz et al., 2016). In recent years, several mulching techniques have been developed and adopted, including (1) flat planting mulched with plastic film (Wang et al., 2011), (2) alternating ridges and furrows with only the ridges mulched with plastic film (Li et al., 2001; Ren et al., 2008), and (3) alternating large and small plastic film-mulched ridges (Liu et al., 2009; Zhou et al., 2009). However, the variable hydrothermal conditions in dryland farming areas mean that different film mulch methods are not equally effective for maize production. Wang et al. (2011) did not find water accumulation from rainfall events < 10 mm when they examined flat planting with maize rows on plastic film mulch without ridges. Li et al. (2001) and Ren et al. (2008) reported greater soil water content in years with different rainfall amounts when plastic film mulch was used in a furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat, bare space (60 cm wide), but the grain yield did not improve. In fact, there was less grain yield in the mulched plots than the unmulched control in a rainy year (annual rainfall > 440 mm) with low temperature because the plastic film mulch trapped precipitation and resulted in high soil water storage levels in the topsoil (0–40 cm). Liu et al. (2009) and Zhou et al. (2009) argued that alternating large and small plastic film-mulched ridges had little or no effect on net income because of high costs and labor inputs, as well increased soil dryness in the deep soil profile with continuous cropping, which caused soil degradation and yield decrease.

Clearly, it is difficult to predict maize productivity in response to plastic film mulch methods, given the diverse responses in crop growth and WUE across the soil hydrological conditions

present in dryland farming areas (Li et al., 2010; Gan et al., 2013), which make difficult to reach the crop productivity potential. Moreover, most previous studies have concentrated on examining the crop yield and soil water effects in farmland exposed to only one plastic film mulch practice (Ren et al., 2008; Zhou et al., 2009; Zhang et al., 2011; Gan et al., 2013). There is scant information to compare among plastic film mulch practices when cultivating the same crop under same agro-ecological conditions. With the film mulch gradually popularized in semiarid area, it is necessary to compare and then find a more sustainable and economic methods for alleviating drought stress and increasing crop yield in these regions. Therefore, in this study, we explored the effects of three different present plastic film mulch practices on spring maize production under the same cultivation conditions.

In addition, several disadvantages of plastic film mulch practices are known, i.e., placing plastic film mulch on a flat planting without ridges is ineffective to accumulate rainfall; the wide furrow covered with plastic film mulch tends to be cooler, which delays crop growth and development; it is expensive to use plastic film to mulch alternating large and small ridges, and this practices may deplete deep soil water reserves. Thus, it is necessary to test a new film mulch technique to alleviate the deficiencies of the current plastic film mulch techniques. Therefore, a new practice where consecutive plastic film-mulched ridges (each ridge measures 50 cm wide) are separated by planted furrows (each 10 cm wide without plastic film mulch) as an alternative configuration was tested in this study. The objectives of our research were: (i) to assess the effects of different plastic film mulch practices on soil water storage and temperature levels and its distribution in soil profile during the maize growing season; (ii) to explore the influence of our new plastic film mulch practice on grain yield, WUE and economic benefits in semiarid regions of the Loess plateau, China.

MATERIALS AND METHODS

Site Description

The field experiments were conducted during 2013 and 2014 at the Dryland Agricultural Research Station, Pengyang County, Ningxia, China (106°45'N, 35°79'E and 1800 m a.s.l.). The experimental area is characterized by a semiarid, warm temperature, and continental monsoon climate. The average annual precipitation was 440 mm, and in this region ranges from 150 and 300 mm in the north to 500–700 mm in the south, more than 60% of which occurred from July to September. The annual mean temperature average was 8.1°C and the annual mean evaporation was 1100 mm, with a frost-free period of 158 days.

The field experimental was conducted on a flat field. According to the FAO/UNESCO Soil Classification (FAO/UNESCO, 1993), the soil at the experimental site was a Calcic Cambisol (sand 14%, silt 26%, and clay 60%) with relatively low fertility. Selected soil physico-chemical properties at the beginning of the experiment are presented in **Table 1**.

TABLE 1 | Selected physico-chemical properties of the loess soil (0–60 cm depth) at the Dryland Agricultural Research Station, Pengyang County, China.

Soil layer (cm)	Organic matter (g kg ⁻¹)	Available nitrogen (mg kg ⁻¹)	Available phosphorus (mg kg ⁻¹)	Available potassium (mg kg ⁻¹)	Total nitrogen (g kg ⁻¹)	Bulk density (g cm ⁻³)	Porosity (%)	Saturated moisture (%)	pH
0–20	8.65	63.6	12.6	161.2	1.19	1.33	49.8	37.4	8.4
20–40	7.95	44.9	7.9	117.2	0.94	1.34	49.4	36.4	8.5
40–60	7.57	46.8	6.0	102.7	1.05	1.41	46.8	38.4	8.6

Organic matter was determined using the Walkley–Black method; The total nitrogen was determined by Kjeldahl digestion; The available phosphorus was determined using the molybdenum blue method; The available nitrogen was determined by alkaline hydrolysis method; Available potassium was determined with flame photometric method; The soil bulk density was determined using the core method, and soil porosity was calculated according to bulk density; pH was determined by potentiometry method.

Experimental Design and Field Management

The experiment was a completely randomized block design with three replicates of five treatments. Plots measured 3.6 m wide and 11.0 m long and were under conventional tillage. The plots were under conventional tillage. As shown in **Figure 1**, treatments were: (i) the control, conventional flat planting without plastic film mulch (CK); (ii) the flat planting with maize rows (60 cm spacing) on plastic film mulch (PM), where the flat planting and plastic film mulch area measured 70 cm wide, with a 50 cm-wide un-mulched space between the two rows of mulched film, and maize was sown as a double row in the film; (iii) furrow planting of maize, separated by consecutive plastic film-mulched ridges (FCM), where the ridges were 50 cm wide and 15 cm high, which were covered with plastic film (70 cm wide), and the furrows were 10 cm wide for sowing a single row of maize; (iv) furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (FLSM), where large ridges (70 cm in width by 15 cm in height) were alternated with small ridges (50 cm in width by 10 cm in height) and both were mulched with plastic film measuring 140 cm wide, and the two ridges were separated a furrow in which the maize was planted; and (v) furrow-flat planting of maize with a large plastic film-mulched ridge alternating with a flat without plastic film-mulched space (FLM), where the ridges covered with plastic film measured 60 cm wide and 15 cm high, and the furrows without plastic film mulch were both 60 cm wide for sowing double rows of maize in the film-side.

The plastic film was polyethylene with a thickness of 0.008 mm, which was made by the Gansu Tianbao Plastic Plant, China, and the plastic film was stability and not decomposed after crop harvested. A sketch of each plastic film mulch mode is presented in **Figure 1**.

The experimental plots were established in March 24, 2013 by plowing the field and delineating the plots. Ridges were formed in 9 of the 15 plots. Ten days before planting, basal fertilizers (150 kg N ha⁻¹ and 150 kg P₂O₅ ha⁻¹) were applied across the unridged plots (six plots: CK and PM treatments) and incorporated manually with a spade to 5 cm depth, or spread in the furrow (nine plots: FLSM, FLM and FCM treatments) and mixed manually to a depth of 5 cm with a spade. Plastic film mulch was placed on the soil surface according to the configurations in **Figure 1** within 2 days after fertilization.

Maize (Dafeng 30) was sown at a rate of 75 000 plants ha⁻¹ on April 14, 2013 using a hole sowing (3 cm in diameter) machine. In addition, 150 kg N ha⁻¹ was applied as a top dressing in late June after maize planting. Crops were harvested from the plots on September 28, 2013. After harvesting the maize, the configuration and mulch were retained in the same location on each of the plots, but the maize stalks were removed and the plastic film was cleared up to 30 days before subsequent sowing operation (March 27, 2014), corn planting in April 28, 2014, the post-emergence fertilization on June 29, and harvest on October 4, 2014, while the process and method was similar as that in 2013. Artificial irrigation was not provided throughout the years of the experiment and weeds were controlled manually during each crop growth season, as required.

Sampling and Measurement

During the experimental period, rainfall data were recorded using an automatic standard weather station (WS-STD1, Delta-T, UK) located at the experimental site.

Mercury-in-glass geothermometers (Hongxing Thermal Instruments, China) were placed between the two maize plants in each treatment plots at soil depths of 5, 10, 15, 20, and 25 cm to determine the soil temperature. Soil temperatures were recorded at 08:00, 10:00, 12:00, 14:00, 16:00, 18:00, and 20:00 h each day at 10, 40, 70, 100, 130, and 170 days after planting. Mean daily soil temperature was calculated as the average of readings of 3 days.

Soil water content was determined at 20 cm increments, to a depths of 200 cm at 10, 40, 70, 100, 130, and 170 days after planting. A 54 mm diameter steel core-sampling tube was inserted manually between two plants, located in the middle rows of each plot, at three locations per plot. Soil cores were weighed wet, dried in a fan-assisted oven at 105°C for 48 h, and the dry weighed assessed to determine the soil water content (Ferraro and Ghersa, 2007). The gravimetric water content was multiplied by soil bulk density to obtain the volumetric water content.

The soil water storage was calculated using Eq (1) as follows:

$$S_w = h \times d \times b\% \times 10 \quad (1)$$

where S_w (mm) is the averaged values of soil moisture; h (cm) is soil layer depth; d (g cm⁻³) is soil bulk density in different soil layer, and $b\%$ is the percentage of soil moisture in weight.

In 2013 and 2014, 30 representative maize plants per plot were used for each measurement at harvest, whilst the ear length,

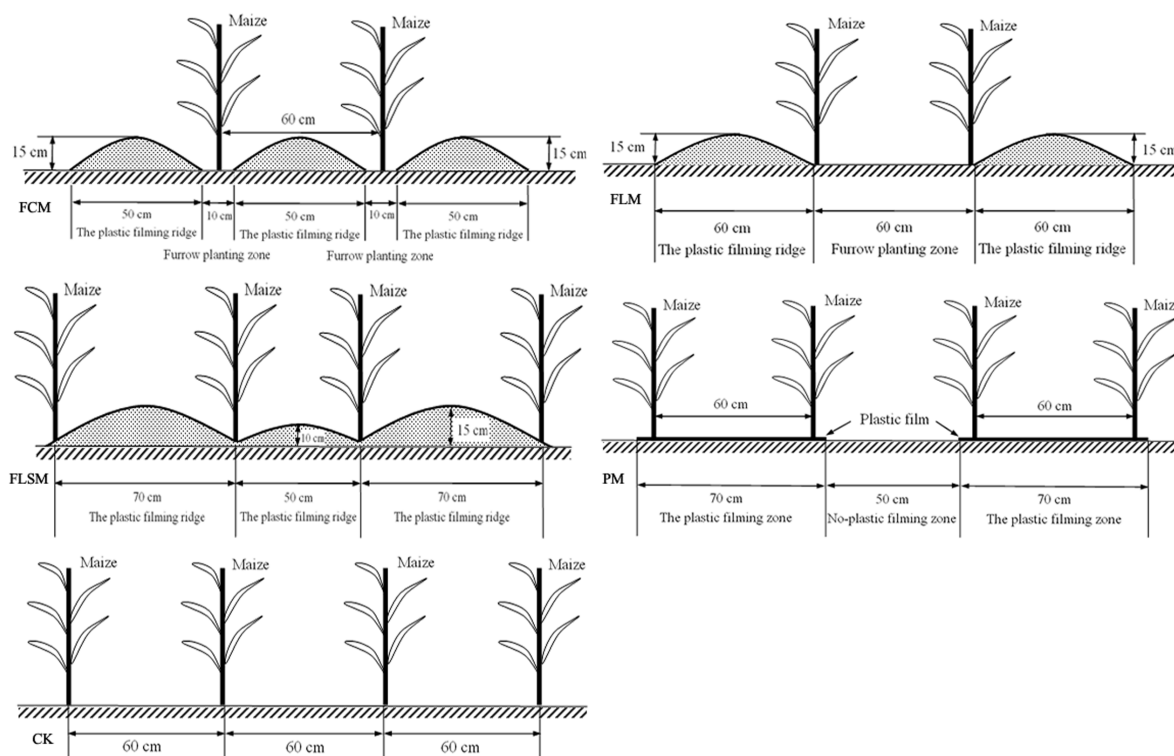


FIGURE 1 | Schematic diagram of the field layout. CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide).

ear diameter, seed number per ear, and 100-kernel weight was recorded.

The WUE was estimated as the grain yield divided by the growing season evapotranspiration (ET, mm) (Hussain and Al-Jaloud, 1995), as follows:

$$WUE = \text{Yield}/ET \quad (2)$$

where ET was calculated as (Li et al., 2013):

$$ET = W_1 - W_2 + P \quad (3)$$

where W_1 (mm) is the soil water storage for the 0–200 cm soil depth before sowing, W_2 (mm) is the soil water storage for the 0–200 cm soil depth at harvesting, and p (mm) is the rainfall during the maize growing season.

The harvest index (HI) based on maize grain yield and biomass yield was calculated as follows:

$$HI = \frac{Y_g}{Y_b} \quad (4)$$

where Y_g (kg ha^{-1}) is the grain yield, and Y_b (kg ha^{-1}) is the biomass yield.

Net economic profit for each treatment was calculated using the following equations:

$$OV = Y_g \times P_g + Y_b \times P_b \quad (5)$$

$$IV = LC + MC + MCC + SFC \quad (6)$$

$$O/I = \frac{OV}{IV} \quad (7)$$

$$NI = OV - IV \quad (8)$$

where OV is the output value (Chinese Yuan ha^{-1}), Y_g (kg ha^{-1}) is the grain yield, Y_b (kg ha^{-1}) is the biomass yield, P_g and P_b is the local price of maize grain and biomass (Chinese Yuan ha^{-1}), IV is the total input value (Chinese Yuan ha^{-1}), LC is the labor cost (Chinese Yuan ha^{-1}), MC is the film mulching cost (Chinese Yuan ha^{-1}), MCC is the machine-cultivation cost (Chinese Yuan ha^{-1}), SFC is the seed and fertilizer cost (Chinese Yuan ha^{-1}), and NI is the net income (Chinese Yuan ha^{-1}).

Statistical Analysis

Data values were analyzed by residual test method before statistical analysis, and the data met the assumption of

homogeneity of variances and followed the normal distribution. Significant differences were determined by ANOVA, and multiple comparison analysis were performed with Tukey HSD test ($p < 0.05$). All the analyses were performed with a confidence level of 95% by using SPSS 13.0 (SPSS Inc. Chicago, IL, USA). All figures were prepared using Sigma Plot 12.5.

RESULTS

Rainfall

Precipitation during the maize growing seasons was 594 in 2013 and 342 mm in 2014, while the 20-year average was 339 mm (Figure 2), indicating that 2013 was a wetter-than-normal season while 2014 was a normal season. It also showed that the rainfall was erratic and has different patterns each year, but temperature pattern was relatively consistent during the two growing season (Figure 2).

Soil Temperature

Soil temperature at 5 cm depth was significantly greater in plots with plastic film mulch than the control plots during early maize growth (up to 40 DAP) by as much as 1.9°C in 2013 and 1.7°C in 2014 (Figure 3). As the maize canopy developed during the growing season, the soil temperature was cooler in the plastic film mulch plots, and the mean soil temperature of at 5 cm depth of plastic film mulch plots was lower than CK (after to 130 DAP) by 1.5°C in 2013 and 2.1°C in 2014. At all growth stages, soil temperature at 10 and 15 cm depth was warmer in the FLSM treatment than the CK (up to 2.1°C in 2013 and 2.6°C in 2014) and in the PM treatment than the CK (by as much as 1.5°C in 2013 and 2.2°C in 2014). Soil temperature at 10 and 15 cm depths was warmer in the FCM and FLM treatments than the CK from 0 to 70 DAP, and there after the temperature was similar in these treatments. During the growing season, soil temperature at 20 cm depth was greater in plastic film mulch treatments than the CK plots, by as much as 0.6°C in 2013 and 1.6°C in 2014, and we also recorded higher soil temperature at 25 cm depth with plastic film mulch than the CK, up to 1.2°C warmer in 2013 and 1.8°C hotter in 2014.

Soil Water Storage

Plastic film mulch improved soil water storage during the early maize growth. From 0 to 10 DAP in 2013, based on statistical analysis (Turkey test), the soil water storage in the 0–60 cm depth was greater in plots with plastic film mulch than the CK plots by as much as 11.7% (FCM treatment, $p < 0.05$), 10.7% (FLM treatment, $p < 0.05$), 7.5% (FLSM treatment), and 7.2% (PM treatment), respectively (Figure 4). Soil water reserves were replenished by 121.4 mm rainfall during April to May, and depleted by maize water use, which resulted in no significant difference among the treatments at 40 DAP. Only FCM and FLM had significantly ($p < 0.05$) more soil water storage than the CK (8.2 and 9.5%, respectively) at 70 DAP. From 100 to 130 DAP, several rainfall events delivered more than 260 mm of precipitation (45% of annual rainfall) which replenished the soil water reserves, and resulted in 7.1% more soil water storage, on

average, in the plastic film mulch treatments than the CK by 170 DAP.

From 0 to 10 DAP in 2013, based on statistical analysis (Turkey test), the soil water storage in the > 60–120 cm depth was significantly ($p < 0.05$) greater in plots with plastic film mulch than the CK plots by 15.0%. Soil water reserves were depleted by maize water use, which resulted in only FLM and FCM had significantly ($p < 0.05$) more soil water storage than the CK (12.1 and 14.7%, respectively) at 40 DAP. Only FLM had significantly ($p < 0.05$) more soil water storage than the CK by as much as 7.9% at 70 DAP, and 4.2% at 100 DAP. At 130 DAP, the soil water storage in the >60–120 cm depth with plastic film mulch than the CK plots by 7.8%. Soil water reserves were replenished by rainfall increase and consumption decrease, and resulted in 6.2% more soil water storage, on average, in the plastic film mulch treatments than the CK at 170 DAP.

The trend of soil water storage of each plots at > 120–200 cm depth increased at 0–100 DAP and then decreased (Figure 4). From 0 to 100 DAP in 2013, only FLM and FCM had more soil water storage than the CK by as much as 3.8 and 5.9% ($p < 0.05$), respectively. At 130 DAP, all plastic film mulch plots was lower than CK by 4.1%, and no significant difference among the treatments at 170 DAP.

Less rainfall during the maize growth stage, which resulted in the soil water storage in 2014 was lower than that in 2013 at each soil depth (Figure 4). Plastic film mulch improved soil water storage during the early maize growth. Based on the analysis of statistical results, in 2014, the soil water storage in the 0–60 cm depth was greater in plots with plastic film mulch than the CK plots by as much as 21.4% ($p < 0.05$) at 10 DAP, 15.1% ($p < 0.05$) at 40 DAP, and 5.9% at 100 DAP. Soil water reserves were replenished by 63.6 mm rainfall during 100–130 DAP, which resulted in only FLM and FCM had significantly ($p < 0.05$) more soil water storage than the CK (19.2 and 16.2%, respectively). At 170 DAP, 146.3 mm rainfall of precipitation was replenished the soil water reserves, and resulted in 9.2% more soil water storage, on average, in the plastic film mulch treatments than the CK.

From 0 to 40 DAP, the soil water storage in the >60–120 cm depth was significantly ($p < 0.05$) greater in plots with plastic film mulch than the CK plots by 12.4%. Soil water reserves were depleted by maize water use, which resulted in all plastic film mulch plots was lower than CK by 9.6% at 100 DAP. At 130 DAP, all plots soil water storage was lowest (range 100–120 mm), and no significant difference among the treatments. Soil water reserves were replenished by rainfall, and resulted in only FLM and FCM had more soil water storage than the CK (2.3 and 9.3%, respectively) at 170 DAP.

Plastic film mulch decreased soil water storage during the late maize growth. From 70 to 170 DAP in 2014, the soil water storage in the > 120–200 cm depth was lower in plots with plastic film mulch plots w than the CK by 18.3%, no significant difference among the treatments.

Crop Development

Early maize growth was accelerated with plastic film mulch treatments compared to the CK in both years (Table 2). This

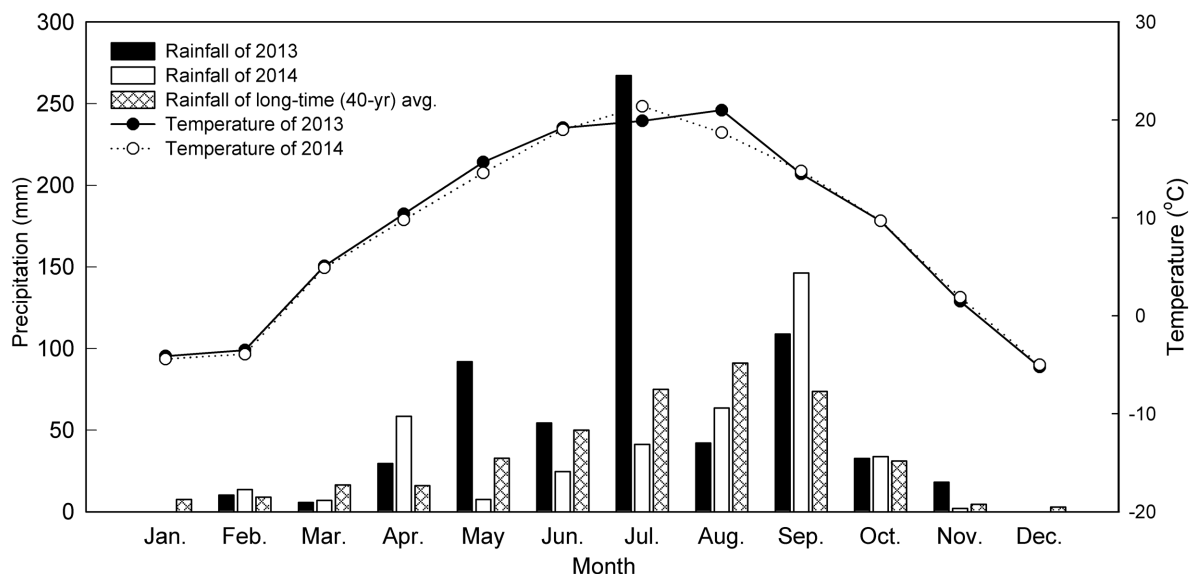


FIGURE 2 | Distribution of monthly precipitation and air temperature during 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China.

resulted in a shorter time to physiological maturity, from 10–16 days in 2013 and 5–12 days in 2014. Plastic film mulch treatments advanced the sowing-emergence time by 3–8 days in 2013 and 4–8 days in 2014. Similarly, the emergence-jointing stage was 4–8 days earlier in 2013 and 3–7 days earlier in 2014. The jointing-tasselling stage was also advanced, by 6–9 days in 2013 and 3–9 days in 2014. As a result, the milk-maturity stage was 5–12 days longer in 2013 and 7–14 days longer in 2014.

Agronomic Properties

Maize grown in plastic film mulch treatments had bigger ears, which were from 5.8 to 9.0% longer and had a 8.1–12.9% larger diameter than those from the CK plots, based on maize samples collected during the 2013 and 2014 growing seasons (Table 3). Based on statistical analysis (Turkey test), grain weight increased significantly ($p < 0.05$) when maize was grown in plastic film mulch, and the 100-kernel weight was 23% greater with FCM, 16% higher with FLM, 24% more in FLSM, and 5.9% greater with PM than the CK plots (Table 3). Similarly, the grain number per ear and shelling percentage of maize were improved significantly ($p < 0.05$) when maize was grown on plastic film mulch (on average, 5.4% more grains per ear and 4.5% higher shelling percentage) than in the CK plots.

Yield and Water Use Efficiency

Grain and biomass yield of maize was significantly influenced by the different plastic film mulch plots over the 2 years, with the higher yield recorded in 2013 and the lower in 2014 (Table 4). In 2013, the maize grain yields for each of the treatments were ranked as follows: FCM > FLSM > PM > CK > FLM, and the results of the statistical analysis (Turkey test) showed that the maize yield with FCM, FLSM, and PM treatments were significantly ($p < 0.05$) higher than CK by 20.3, 18.0, and 11.2%,

respectively. While in 2014, the yields were ranked as follows: FLSM > FCM > FLM > PM > CK, the mean maize yields with plastic film mulch plots were significantly ($p < 0.05$) higher than CK by 34.9%.

The biomass yields have the similar trends with the grain yield. Based on statistical analysis (Turkey test), the mean biomass yield with plastic film mulch (except FLM) was significant higher than CK by 6.3% in 2013 and 19.7% ($p < 0.05$) in 2014.

The HI was ranging from 0.57 and 0.67 in 2013, only FCM significantly higher than CK (15.5%). While in 2014, the results of the statistical analysis (Turkey test) showed that the plastic film mulch plots (except FCM) were all significantly ($p < 0.05$) higher than CK, i.e. the FLM, FLSM, and P treatments significantly increased by 18.8, 10.4, and 33.3%, respectively.

The WUE have the similar trends with the yield. Based on statistical analysis (Turkey test), the WUE of plastic film mulch plots (except FLM) were all significantly higher than CK in 2013, i.e., significantly ($p < 0.05$) increased by 13.3, 13.4, and 21.2% with FCM, FLSM, and PM, respectively. In 2014, the WUE was greater in plots with plastic film mulch than the CK plots by 26.6%.

Because of the difference of rainfall, the ET of each treatment was higher in 2013 than in 2014. In 2013, only FCM significant ($p < 0.05$) increased by 6.3% compared with CK. While in 2014, the plastic film mulch plots were all higher than CK, average increased by 6.7%.

Economic Benefit

There were obvious differences in the input costs of the various plastic mulch plots, because of the use of mulching amounts and labor (Table 5). The 2-year average input cost was ranked as follows: FLSM > FCM > PM > FLM > CK, while the output value followed by FLSM > FCM > FLM > PM > CK. The

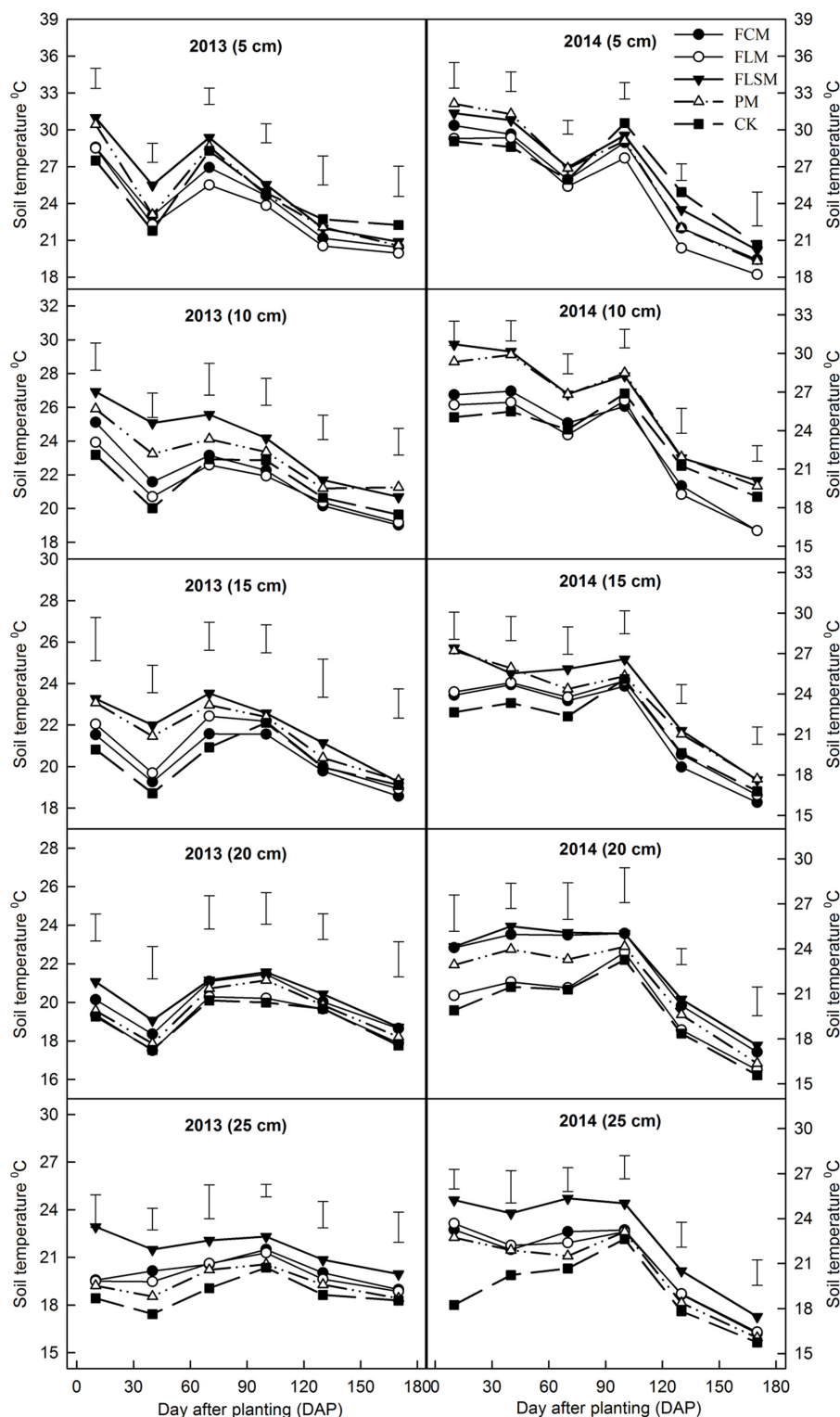


FIGURE 3 | Effects of different film mulching treatments on soil temperature at different soil depths and times in 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China. CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide). Error bars indicate 1 s.d. value.

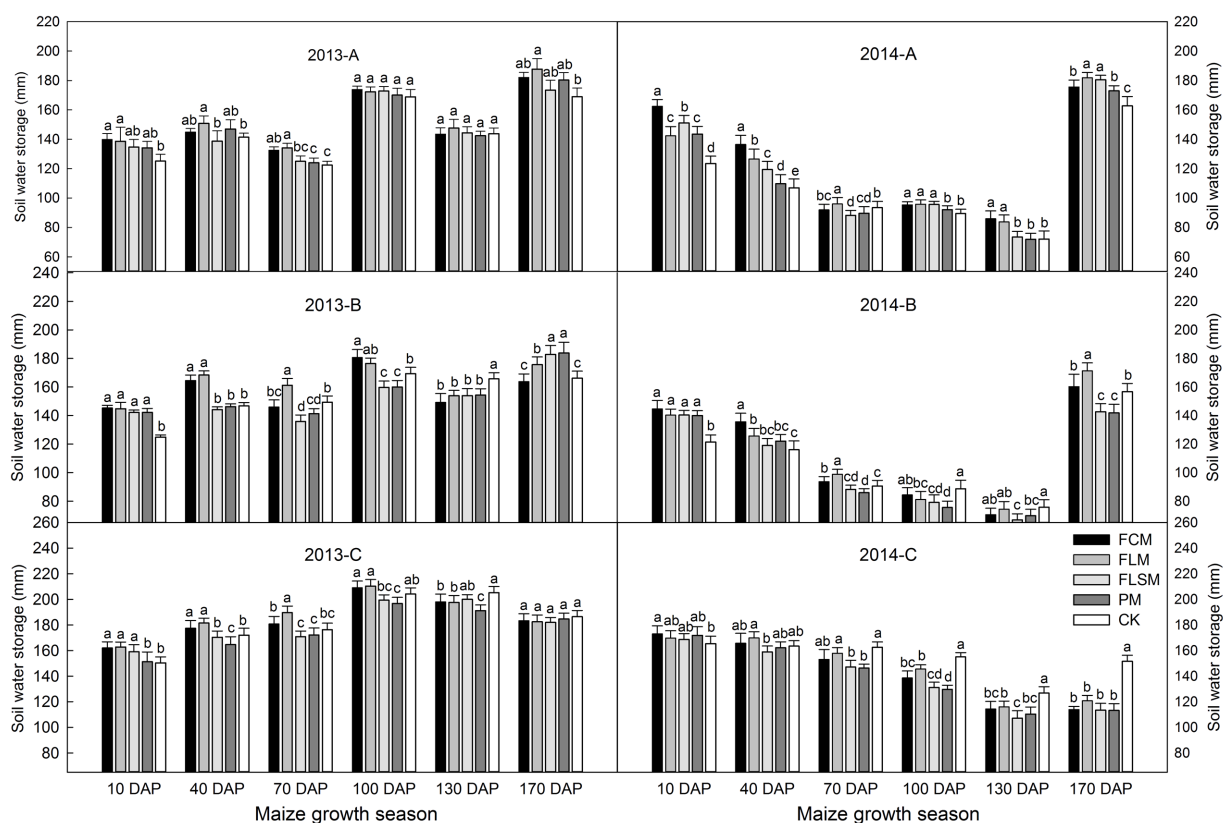


FIGURE 4 | The soil water storage dynamics in 0–60, 60–120, and 120–200 cm layers with different film mulching treatments during maize growing season in 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China. (A–C) Soil water storage at 0–60 (A), 60–120 (B), and 120–200 (C) cm layers, respectively. CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM: furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide). Data are means \pm SD ($n = 3$). Bars with different lower case letters indicate significant differences among treatments for each year (Tukey HSD test, $p < 0.05$).

TABLE 2 | Maize crop development (day) under different mulch plots during 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China.

Year	Treatments	Sowing-Emergence	Emergence-Jointing	Jointing-Trumpeting	Trumpeting-Tasseling	Tasseling-Blooming	Blooming-Milking	Milking-Maturity	Total
2013	FCM	7	28	21	21	4	7	65	153
	FLM	10	30	20	22	4	10	59	155
	FLSM	5	26	20	20	4	8	66	149
	PM	5	28	18	21	4	11	65	152
	CK	13	34	23	25	5	11	54	165
2014	FCM	8	37	17	19	3	6	56	146
	FLM	10	41	18	21	4	8	51	153
	FLSM	6	39	16	17	3	7	58	146
	PM	8	39	17	17	3	6	56	146
	CK	14	44	20	22	4	10	44	158

CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide).

TABLE 3 | Effects of different mulch plots on agronomic properties of maize during 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China.

Year	Treatments	Ear length (cm)	Ear diameter (cm)	100-kernel weight (g)	Grain number per ear	Shelling (%)
2013	FCM	20.19a	51.42ab	42.56a	608.48a	86.30a
	FLM	18.73b	49.04b	39.73ab	585.79bc	82.58b
	FLSM	19.26ab	51.82a	42.15a	595.96abc	84.10ab
	PM	19.04b	50.23ab	35.19c	605.31ab	86.11a
	CK	18.28b	49.54ab	36.03b	582.71c	81.91b
2014	FCM	19.52a	52.38ab	33.73a	640.13a	80.38a
	FLM	20.41a	51.68ab	32.50ab	631.33a	81.31ab
	FLSM	20.10a	52.75a	34.85a	633.09a	82.82a
	PM	19.49a	50.40b	30.55b	578.39b	80.40b
	CK	18.15a	46.34c	26.07c	574.35b	77.01c

CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide). Values followed by the different lowercase letter in the same row indicate significant differences among treatments for each year (Tukey HSD test, $p < 0.05$).

TABLE 4 | Effects of different mulch plots on the grain yield, biomass yield, harvest index (HI), evapotranspiration (ET), and water use efficiency (WUE) of maize in 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China.

Year	Treatments	Grain yield (kg ha ⁻¹)	Biomass yield (kg ha ⁻¹)	HI	ET (mm)	WUE (kg ha ⁻¹ mm ⁻¹)
2013	FCM	16200a	24422ab	0.67a	522.02a	31.09a
	FLM	12957c	22769b	0.57b	509.66a	25.42b
	FLSM	15896a	25271a	0.63ab	505.38a	31.11a
	PM	14976b	25031ab	0.60ab	450.17b	33.27a
	CK	13470c	23432ab	0.58b	490.91a	27.44b
2014	FCM	13509a	24359ab	0.46d	475.86bc	28.39a
	FLM	13421a	23761ab	0.57b	478.90ab	28.12a
	FLSM	13671a	26242a	0.53bc	496.12ab	27.55a
	PM	11169b	20986bc	0.64a	500.59a	22.32b
	CK	9594b	19917c	0.48cd	457.06c	21.01b

CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM, furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide). Values followed by the different lowercase letter in the same row indicate significant differences among treatments for each year (Tukey HSD test, $p < 0.05$).

output/input ratio of each plastic film mulch plots was lower than CK (average decreased by 15.2%). Net income of FCM, FLM, FLSM treatments were higher than that of CK, i.e., increased by 1559, 528, and 350 CNY ha⁻¹, respectively. While PM treatment was lower than CK by 538 CNY ha⁻¹.

DISCUSSION

Field management practices affect the soil surface conditions as well as influencing the soil water and thermal status, which play important roles in crop growth and development during dryland farming (Chakraborty et al., 2008). In the loess plateau region of northwest China, intensive cultivation systems are employed but with poor soil management strategies (Wang et al., 2009; Zhang et al., 2009). The results of the present study demonstrate that plastic film mulch had positive effects on the soil water storage, soil temperature, and crop yield.

Therefore, appropriate plastic film mulch managements are very important for sustainable agricultural development in these semiarid areas, and also applied to other similar regions of the world.

Soil Temperature

A suitable soil temperature is a basic requirement for crops to maintain the root activity, while changes in the root morphology may affect crop yield (Stone et al., 1999). Several studies have shown that suitable temperatures during the early stage of crop growth can greatly accelerate grain germination and crop yield (Ren et al., 2016). Our results showed that irrespective of depth, the effects of different plastic film mulch plots on the soil temperature were greater during the early growth stages, with a mean increase of 0.8°C, which was probably because the plant canopy was sufficiently small and sparse during the early stage of crop development so the majority of the plastic film area received solar energy to warm the topsoil (Liu et al.,

TABLE 5 | Average economic output and input costs for maize production during 2013–2014 at the Dryland Agricultural Research Station, Pengyang County, China.

Treatments	LC	MC	MCC	SFC	IV	OV	O/I	NI	NID
FCM	2700	1800	1500	3135	9135	20264	2.22	11129	1558.6
FLM	2700	720	1500	3135	8055	18153	2.25	10098	527.5
FLSM	3600	2160	1500	3135	10395	20316	1.95	9921	350
PM	3600	720	1500	3135	8955	17988	2.01	9033	−538
CK	1800	0	1500	3135	6435	16006	2.49	9571	0

CK, the control, conventional flat planting without plastic film mulch; PM, flat planting with maize rows (60 cm spacing) on plastic film mulch (70 cm wide); FCM, furrow planting of maize (60 cm spacing), separated by consecutive plastic film-mulched ridges (each 50 cm wide and 15 cm tall); FLSM, furrow planting of maize (60 cm spacing), separated by alternating large and small plastic film-mulched ridges (large ridges: 70 cm wide and 15 cm tall, small ridges 50 cm wide and 10 cm tall); FLM: furrow-flat planting of maize (60 cm spacing) with a large plastic film-mulched ridge (60 cm wide and 15 cm tall) alternating with a flat plastic film-mulched space (60 cm wide). LC, labor costs [Chinese yuan (CNY) ha^{−1}]; MC, film mulching costs (CNY ha^{−1}); MCC, machine-cultivation costs (CNY ha^{−1}); SFC, seed and fertilizer costs (CNY ha^{−1}); IV, input value (CNY ha^{−1}); OV, output value (CNY ha^{−1}); O/I, output/input; NI, net income (CNY ha^{−1}); NID, net income difference (CNY ha^{−1}) compared with CK. Labor cost = 80 CNY per person per day; plastic film cost = 12 CNY kg^{−1}; maize seed price = 1.20 CNY kg^{−1}; maize straw price = 0.1 CNY kg^{−1}.

2010; Gan et al., 2013). It was shown that the plastic film mulch could provide a favorable soil temperature for crop emergence. By contrast, after the full establishment of the plant canopy during the middle and later growth stages, the soil temperature increased little under plastic film mulch compared with the uncovered plots (CK), and because of the higher soil water storage caused low soil temperature, the FLM and FCM plots lower than CK in the 5–15 cm soil depth at 70 DAP, because the FLM and FCM treatments accumulated rainwater by ridge and furrow systems. We also showed that the effects of FLM and FCM on soil temperature increasing was significant in deeper (20–25 cm depth) layers compared with the surface layers of the soil (**Figure 3**), which agreed with the findings of Ren et al. (2008) and Li et al. (2013). This support a favorable soil microclimate for maize root proliferations to increasing soil WUE (Osuji, 1990). By contrast, the soil temperature was consistently higher in each soil layer with FLSM and PM, because the film covered the entire maize planting area, thereby preventing water exchange between the soil and air to reduce the latent heat flux (Liu et al., 2009, 2010), which led to the crop grew quickly and consumed lots of soil water to destroyed soil water balance.

Soil Water Storage

Many studies have indicated that plastic film mulch could significantly reduce soil water evaporation and water erosion, thereby increasing the precipitation use efficiency in rain-fed farming systems (Ramakrishna et al., 2006; Turner et al., 2011; Gan et al., 2013). If the soil is dry during the seedling stage, the seeds cannot absorb sufficient water and germination may be impossible or delayed, while root productions might be decreased after seed germination, thereby affecting aboveground growth and seed yield (Ren et al., 2016). Our results showed that the plastic film mulch plots significantly increased the soil water storage (0–200 cm) compared with CK during the early growth stage (0–40 DAP), especially in FLM and FCM plots, and similar results were also reported by Li et al. (2013). This is mostly because the plant canopy is small in the early stage, and there is high water evaporation from the bare soil, but plastic film can significantly prevent the soil water exchange between the soil and air to decrease the evaporation of soil

water, which provided more favorable condition for seedling growth by spring crops in the test area where the average annual temperature was only 8.1°C (Ramakrishna et al., 2006). In our research, during elongation in the maize growth stage, the soil water storage of FLSM/PM plots was lower than FLM/FCM at 0–60 cm depth, especially in the year with less rainfall (2014). There are two possible explanations for this difference: plastic film mulch on all maize grow area produced a higher soil temperature so the plants grew quickly and consumed more soil water; or most of the rainfall occurred as light rains (<10 mm) during this stage and 85% of the precipitation was lost as runoff (Ren et al., 2009), the ridge and furrow system (FLM and FCM treatments) using mulching ridges to accumulated rainwater, which increased the penetration of light rain into deep soil (Li and Gong, 2002; Tian et al., 2003). Moreover, we showed that the soil water storage in the deeper soil layer (>60–200 cm) was lower under the plastic film mulch plots than CK when the maize entered the reproductive stage, probably because most of water consumed in these stages for maize growth come from the deeper soil layer (Gan et al., 2013), and the abundant rainfall during the reproductive stage (more than 70% of the growing season rainfall occurs from July to September) could not infiltrate to the deeper layers rapidly, which also led to water deficit in the deeper layers under plastic film mulch plots.

Ren et al. (2008) observed that the most obvious effects of the ridge and furrow system on the soil water storage level occurred with annual precipitation between 230 and 440 mm, whereas there were no significant effects when the rainfall exceeded 440 mm. Li et al. (2001) also found that the ridge and furrow system decreased the soil water storage level as the precipitation increased, which agreed with our results. We found that the FLM and FCM treatments significantly increased the soil water storage in the 0–120 cm layer compared with FLSM and PM in 2014, which agreed with the results reported by Ren et al. (2010), who showed that the ridge and furrow system concentrates the rainfall and forced deeper penetration in the soil to reduce evaporation but also lateral moved into the ridges to retain soil water. In addition, it is possible that FLSM and PM consumed more soil water than FLM and FCM caused by quickly crop development.

Crop Development

Previous studies have demonstrated that plastic film mulch can increase the soil temperature (Li et al., 2013) and soil water content (Li et al., 2001), thereby reducing germination time and promoting crop growth and development to increase grain yield. Similarly, we found that the plastic film mulch plots clearly increased soil temperature in early stage, and advanced the emergence and maturity stages compared with CK. The emergence stage under FLM/FCM plots were delayed compared with FLSM/PM by 2–5 days, because the better soil water conditions with FLM and FCM treatments lead to low temperature (Li et al., 2001). In addition, it was also associated with the maize seeding location, planting on plastic film or film-side. The milking-maturity stage was extended with the plastic film mulch plots (especially with FCM, FLSM, and PM), which agreed with the results reported by Liu et al. (2010), who observed that plastic film mulch plots promoted crop transpiration with little soil evaporation to allow the accumulation of a greater biomass during the early growth stages, while the development of plants accelerated from seedling emergence to physiological maturity, and the milking stage extended to increase the maize yield.

Water Use Efficiency (WUE)

It has been widely reported that plastic film mulch can significantly increase the WUE (Raeini-Sarjaz and Barthakur, 1997; Li et al., 2010). Similarly, in our study, the plastic film mulch plots significantly increased the WUE by 10.5–22.8%. Surface plastic film mulch enhances the soil moisture regime by controlling evaporation from the soil surface (Raeini-Sarjaz and Barthakur, 1997; Wang et al., 2009), which improve infiltration and soil water retention, as well as providing a favorable soil microclimate for seedling emergence (Liu et al., 2010), and root proliferation (Osuji, 1990). Under each treatment (except FLM), the WUE was higher in 2013 than 2014 because more abundant rainfall led to significantly higher maize yields in 2013 compared with 2014 (Table 4). In addition, nearly 50% of the rainfall occurred during late-September to the beginning-October in 2014, so it could not be used by the maize crop and it only increased the ET, thereby decreasing the WUE. Our results also demonstrated that the effect of plastic film mulch on the WUE was higher in normal season than a wetter-than-normal season, especially in FLM and FCM plots, which agreed with Li et al. (2001) and Ren et al. (2008). The optimum rainfall amount for ridge and furrow harvesting systems is 230–440 mm and there are no significant improvements in the WUE when the rainfall exceeds 440 mm.

Grain Yield

The better grain yield response of plastic film mulch was largely due to improved topsoil temperature and soil moisture conditions through better utilization of low intensity rainfall (Cook et al., 2006; Li et al., 2008; Ren et al., 2008). Meteorological variations meant that there were differences in grain yield of maize during 2 years. Gan et al. (2013) reported that the yield improvement obtained with plastic film mulch was better when

less rainfall occurred during the growing season. In our research, the grain yield with plastic film mulch were 11.4 and 34.9% higher than CK in 2013 and 2014, respectively. Thus, in a wetter-than-normal year, the soil temperature becomes the key factor that constrains crop growth instead of soil water, and maintain an optimum temperature could produce higher crop yield (Chakraborty et al., 2008). A previous study showed that different planting patterns can affect crop agronomic properties, thereby leading to changes of crop yield in the field (Zhang et al., 2007). The results found that the main increase in the maize yield under plastic film mulch plots was attributable to the higher 100-kernel weight, where this effect was particularly pronounced during the drought or average-rainfall year, i.e., 2014. The rate at which the mulched soil dried was slow and water was conserved at lower depths, and thus the availability of water was maintained for a relatively longer time during the productive growth period, particularly in the milking stage (Zhang et al., 2007; Li et al., 2008). Furthermore, plastic film mulch plots increased the heat available to maize, which is crucial for crop production in semiarid regions (Liu et al., 2010). The yield enhancements differed according to the mulching and configuration of different practice. The FCM plots (furrow planting of maize, separated by consecutive 50 cm wide and 15 cm tall plastic film-mulched ridges) had the best yield increasing effect, an average increased by 3323 kg ha⁻¹ (28.8%) over 2 years. The following better treatment for yield enhancement was obtained for FLSM, and then FLM and PM. This was mainly because when the furrow width was too wide, the soil temperature increased relatively low, and not formed ridges were relatively low amounts of precipitation collected, thereby affecting the maize yield increase. Therefore, the optimum plastic film mulch planting would be FCM (furrow planting of maize, separated by consecutive 50 cm wide and 15 cm tall plastic film-mulched ridges) in the semi-arid dryland agricultural regions, which were characterized by a semiarid (annual rainfall ranges from 300 to 500 mm), warm temperature (annual temperature ranges from 5 to 10°C), and continental monsoon climate.

Economic Benefit

Besides the improvement of yield increasing effect, the economic benefit effect of planting practice is another factor need to be considered. Economic benefit is one of the most effective evaluation indices for crop management practices, which is the most concerned by farmers. The cost of film plots would be higher than CK by about 2700 Chinese Yuan ha⁻¹ (including the costs of labor and the plastic film) every year. The costs of film were higher in FLSM and FCM, because the higher film areas. Notably the labor costs were significantly higher in FLSM and PM, because the FLSM and PM were sowing under the film, and need to releasing seedlings manually, which leads to lower out/put (O/I). However, farmers often give little consideration to the labor cost, which including sowing seed, fertilizing, forming the ridges, mulching, and other field management costs in agricultural production in our experiment area. The film mulch could decrease the infection of diseases and pests in farmland, which also helpful to reduce input value. Plastic film is a relatively low-cost material and many types of plastic film can be readily

found everywhere in the world which will be recycled. In this research, the most important output value from the plots was the maize grain, the market of which price stability between and within seasons in northwest China. The corresponding output value (OV) of plastic film mulch plots would increase about 1982–4310 Chinese Yuan ha^{-1} (maize price is about 1.2 Chinese Yuan kg^{-1}) in average years, especially FCM and FLSM plots. Although the FCM plots needs some investment, it can be offset by growing cash crops, and get a high net income (NI) and highest net income difference (NID), and it is an option with high potential to increase crop sustainability in dryland farming system. This indicated that the FCM methods (furrow planting of maize, separated by consecutive 50 cm wide and 15 cm tall plastic film-mulched ridges) has a great potential to be widely adopted by farmers in the future under semi-arid climate, and it could serve as a new model for spring maize production for small holder farmers in semi-arid regions. However, while producing huge benefits, plastic film mulch technology has also brought on a series of environment pollution hazards. Therefore, we can combine biodegradable film to use FCM method in the future to control residual mulch pollution.

CONCLUSION

The benefits of maize with plastic film mulch in semiarid agricultural systems are enormous, though the effects between plastic film mulch plots varied in different rainfall years. In the present study, mulch with plastic film can inhibit soil evaporation, improve the soil moisture storage, prolong the period of moisture availability, regulating the soil temperature, and promote maize growth, thereby significantly increasing the crop yield and WUE, particular FLSM and FCM treatments. In the long term, the FLSM treatment require high inputs of

money and labor every year, but treatments with FCM (furrow planting of maize, separated by consecutive 50 cm wide and 15 cm tall plastic film-mulched ridges) will bring a significant increase income of farmers. Therefore, this treatment can be an innovative practice in maize production in the rainfed area of the Loess Plateau, China, and also applied to other similar semi-arid dryland agricultural regions of the world.

AUTHOR CONTRIBUTIONS

The manuscript was reviewed and approved for publication by all authors. XR, ZJ, and QH conceived and designed the experiments. PZ, TW, TC, SA, XR, and QH performed the experiments. PZ, TW, TC, and ZJ analyzed the data. PZ, SA, and XR wrote the paper. PZ, TW, TC, SA, QH, XR, and ZJ reviewed and revised the paper. SA, TW, and ZJ corrected the English language for the paper.

FUNDING

This study was supported by the China Support Program (2012BAD09B03) for Dryland Farming in the 12th 5-year plan period, the National High-Tech Research and Development Programs of China (“863 Program”) for the 12th 5-Year Plans (2013AA102902), the China Postdoctoral Science Foundation funded project (2016M602870), the Program of Introducing Talents of Discipline to Universities (No. B12007).

ACKNOWLEDGMENT

We are also grateful to Wang Junpeng, Nie Junfeng, Yang Baoping and Ding Ruixia for help during experimental period.

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Conflict of Interest Statement: 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.

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Potential of Native Rhizobia in Enhancing Nitrogen Fixation and Yields of Climbing Beans (*Phaseolus vulgaris* L.) in Contrasting Environments of Eastern Kenya

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OPEN ACCESS

Edited by:

Maria Tsiafouli,
Aristotle University of Thessaloniki,
Greece

Reviewed by:

Reinhard Turetschek,
University of Vienna, Austria
Karl Kunert,
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 13 December 2016

Accepted: 14 March 2017

Published: 31 March 2017

Citation:

Koskey G, Mburu SW, Njeru EM, Kimiti JM, Ombori O and Maingi JM (2017) Potential of Native Rhizobia in Enhancing Nitrogen Fixation and Yields of Climbing Beans (*Phaseolus vulgaris* L.) in Contrasting Environments of Eastern Kenya. *Front. Plant Sci.* 8:443. doi: 10.3389/fpls.2017.00443

Climbing bean (*Phaseolus vulgaris* L.) production in Kenya is greatly undermined by low soil fertility, especially in agriculturally prolific areas. The use of effective native rhizobia inoculants to promote nitrogen fixation could be beneficial in climbing bean production. In this study, we carried out greenhouse and field experiments to evaluate symbiotic efficiency, compare the effect of native rhizobia and commercial inoculant on nodulation, growth and yield parameters of mid-altitude climbing bean (MAC 13 and MAC 64) varieties. The greenhouse experiment included nine native rhizobia isolates, a consortium of native isolates, commercial inoculant Biofix, a mixture of native isolates + Biofix, nitrogen treated control and a non-inoculated control. In the field experiments, the treatments included the best effective native rhizobia isolate ELM3, a consortium of native isolates, a commercial inoculant Biofix, a mixture of native isolates + Biofix, and a non-inoculated control. Remarkably, four native rhizobia isolates ELM3, ELM4, ELM5, and ELM8 showed higher symbiotic efficiencies compared to the Biofix. Interestingly, there was no significant difference in symbiotic efficiency between the two climbing bean varieties. Field results demonstrated a significant improvement in nodule dry weight and seed yields of MAC 13 and MAC 64 climbing bean varieties upon rhizobia inoculation when compared to the non-inoculated controls. Inoculation with ELM3 isolate resulted to the highest seed yield of 4,397.75 kg ha⁻¹, indicating 89% increase over non-inoculated control (2,334.81 kg ha⁻¹) and 30% increase over Biofix (3,698.79 kg ha⁻¹). Farm site significantly influenced nodule dry weight and seed yields. This study, therefore, revealed the potential of native rhizobia isolates to enhance delivery of agroecosystem services including nitrogen fixation and bean production. Further characterization and mapping of the native isolates will be imperative in development of effective and affordable commercial inoculants.

Keywords: native rhizobia, ecosystem services, biological nitrogen fixation, climbing beans, Eastern Kenya

Abbreviations: AEZ, Agroecological zones; ELM, Embu Lower Midland; EUM, Embu Upper Midland; MAC, Mid-altitude climbers; SEF, Symbiotic efficiency; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland.

INTRODUCTION

Climbing bean (*Phaseolus vulgaris* L.) is one of the most important food crops that is widely cultivated in Sub-Saharan Africa (SSA) and other tropical regions (Ramaekers et al., 2013). The crop is a short-season legume with most varieties maturing between 65 and 110 days after emergence. Production of climbing beans by smallholder farmers is often constrained by the impoverished soil fertility, poor agronomic practices, bean diseases and pest infestation, thus resulting in minimal yields (Beebe et al., 2012). The increasing human population in SSA has led to fragmentation and intensive use of agriculturally prolific lands, leading to exhaustion of available soil nutrients. For instance, each household in Eastern Kenya is estimated to have an average of 0.5–1.0 hectares agriculturally productive farm (Mburu et al., 2016). Limited soil nitrogen has been pointed out as one of the restraining factors in bean production (Shamseldin et al., 2012). To counter soil nitrogen limitation, organic manure, inorganic nitrogen fertilizers and bio-fertilizers are used. However, organic manure is rarely available for smallholder farmers (Gichangi et al., 2012). On the other hand, inorganic nitrogen fertilizers, which boost bean production, are costly and unaffordable to the resource poor smallholder farmers. In addition, the use of inorganic fertilizers has drawn a number of reactions due to negative environmental effects especially on soil biodiversity and aquatic ecosystems (Hester and Harrison, 2012; Mutuma et al., 2014).

Biological nitrogen fixation (BNF) has been widely used as a replacement of nitrogen fertilizers in legume production because of its economic efficiency in the provision of sustainable agroecosystem services (Ouma et al., 2016). Rhizobia are nitrogen-fixing bacteria that live either freely in the soil or form a symbiotic association with the roots of legumes (Martínez-Romero, 2003). Rhizobia are used as bio-fertilizers in legume production and are reported to increase the availability of nitrogen through BNF in different agroecosystems, hence enhancing plant growth and yields (Chabot et al., 1998). Rhizobia biofertilizers are of economic importance in climbing bean production since they offer an alternative farming technology that is eco-friendly, sustainable and enhances soil biodiversity and soil structure (Rahmani et al., 2011). The utilization of native rhizobia as inoculants promote ecologically sustainable management of agricultural ecosystems and enhance legume production due to their growth promoting traits and adaptability to soil and environmental stress (Mwangi et al., 2011). Furthermore, crop production using inoculants could be cheaper and more affordable to the resource-poor smallholder farmers (Singh et al., 2016). The ability of native strains to interact positively with the resident soil microbiota and their adaptability to the local agroecological climatic conditions often elucidates their superior performance over the exotic commercial strains (Meghvansi et al., 2010).

Despite the beneficial effects of rhizobia biofertilizers, it is often necessary to provide legumes with rhizobia inoculants that are infective, and effective in causing nodulation and

nitrogen fixation (Tena et al., 2016). Strain screening and selection is an important step in inoculum development. The existence of native rhizobia isolates that successfully nodulate legumes has been demonstrated in different parts of the world (Anyango et al., 1995; Rahmani et al., 2011; Stajković et al., 2011). Recently, species of the genus *Rhizobium*, such as *R. etli*, *R. giardinii*, *R. leguminosarum*, and *R. tropici* have been reported to nodulate and establish a symbiotic association with different common bean varieties in tropical areas (Torres et al., 2009; Ribeiro et al., 2013). In Kenya, Mathu et al. (2012) demonstrated the potential of native bradyrhizobia to improve cowpea and green gram yields. Variable responses to inoculation using commercial inoculants have been reported and this highlights the need to identify specific native rhizobia strains or their combinations for legume production. The use of host-specific native rhizobia isolates is recommended because they adapt better to the local environmental and soil conditions (Ouma et al., 2016). In addition, native rhizobia isolates are persistent and have better survival rate (Stajković et al., 2011) and this could increase the chances of successful nodulation and nitrogen fixation in the host plant. On the other hand, the inability of introduced commercial inoculants to compete well with native rhizobia population due to negative microbial interactions impedes their use (Martínez-Romero, 2003).

Authentication of rhizobia to determine their symbiotic efficiency is required to screen out effective native rhizobia isolates. This is usually carried out in a greenhouse under bacteriologically controlled conditions (Beck et al., 1993; Maingi et al., 2001). In order to achieve maximum legume productivity, screening of native isolates for their nitrogen fixation efficiencies is vital (Anglade et al., 2015). Furthermore, screening is important in the development of effective legume inoculum. Limited information on the symbiotic nitrogen-fixing potential of the mid-altitude climbers (MAC) with native rhizobia isolates in contrasting environments of Eastern Kenya is available. Previous studies have majorly focused on nitrogen fixation potential of bush beans, and traditional climbing bean varieties and their yield performance in different cropping conditions (Kimani et al., 2007; Gicharu et al., 2013; Ouma et al., 2016). The results from this study will contribute toward the development of rhizobia inoculum for use in the production of common beans in different agroecological zones. Moreover, the assessment of native rhizobia and their compatibility with the new bean lines, such as MAC varieties would contribute to the worldwide knowledge of soil microorganisms and their importance in BNF. There is need to identify native rhizobia isolates that nodulate with MAC beans and evaluate their symbiotic efficiency in the greenhouse and their suitability for use as inoculants in the field. The objectives of this study were to evaluate the symbiotic efficiency of native rhizobia isolates and to compare the effect of native rhizobia and commercial inoculants on nodulation, growth and yield parameters of MAC 13 and MAC 64 climbing bean varieties grown in different agroecological zones of Embu and Tharaka Nithi Counties in Eastern Kenya.

MATERIALS AND METHODS

Study Sites

Greenhouse experiments were carried out in the Department of Microbiology, Kenyatta University in Nairobi, Kenya (1°11'10"S, 36°55'30"E). Field experiments were conducted in four selected farms in upper and lower midland agro-ecological zones of Embu and Tharaka Nithi Counties in Eastern Kenya. The experiments were carried out during the long rainy season (March to August 2015) and short rainy season (October to December 2015). The two farms in Embu County (ELM and EUM) are situated at the foot of Mt. Kenya at 0.53° S, 37.45° E within an elevation of 1100 to 1500 m above sea level (a.s.l.) (Jaetzold et al., 2006). The area receives bimodal rainfall pattern with an average of 1500 mm annually. In Tharaka Nithi County, the two farms selected (TLM and TUM) are located on the South-Eastern side of Mt. Kenya at 0.30° S, 38.06° E and lie within an elevation of 600 m to 1500 m a.s.l. (Jaetzold et al., 2006). Tharaka Nithi County receives a relatively lower annual precipitation averaging at 1000 mm annually. The field experimental sites were selected based on agro-climatic conditions and prevalence of climbing bean cultivation. From the two Counties, the farms chosen had no history of rhizobia inoculation. The farm sizes had a characteristic of smallholder farming systems with an average of 0.5–1.0 Ha per household. The dominant crop species in the study area are common beans, maize, cowpeas, soybeans, bananas, tea, and coffee (Mburu et al., 2016). Climbing bean (*Phaseolus vulgaris* L.) varieties (MAC 13-Kenya safi and MAC 64-Kenya mavuno) were used as test plants. They are not only high yielding bean varieties but also heat tolerant and resistant to common bean diseases. The MAC 13 (cream white background with red flecks seeds) and MAC 64 (dark red mottled medium seeded) are suitable for production in an altitude range of 1000–1800 m asl (Ramaekers et al., 2013). The two bean varieties are commercially sold by the Kenya Seed Company Limited (Nairobi).

Soil Sampling and Analyses

Soil sampling was carried out in all the four farms before the onset of long rains (March 2015). The soil was sampled across and diagonally from 20 points in each farm at a depth of 5–20 cm using a hand shovel. A kilogram of a homogeneous composite soil sample was made from each farm and packed independently into sterile bags for laboratory analysis. Soil samples were air-dried and sieved through a 2 mm diameter sieve for physical and chemical analysis. Soil analysis was carried out according to the procedures described by Okalebo et al. (2002).

Rhizobia Trap Cultures

Field trapping of native rhizobia was carried out in all the four farms in Embu and Tharaka Nithi Counties from March to August 2015 using MAC 13 and MAC 64 climbing bean varieties obtained from Kenya Seed Company Limited, Nairobi. The farms were demarcated and prepared for planting by plowing and fine harrowing before the onset of the first rains. Two climbing bean seeds were planted per hill at a spacing of 75 cm by 30 cm. Triple superphosphate (TSP) fertilizers were applied at a rate of

46 kg P₂O₅ ha⁻¹. During the mid-flowering stage, 10 plants from each farm were randomly sampled and harvested by carefully excavating the root systems to recover root nodules. The root nodules showing pink coloration from each bean plant were collected, packed in sterile sampling vials containing cotton wool and silica gel for desiccation. The nodules were transported to the Microbiology laboratory at Kenyatta University where they were air-dried for rhizobia isolation and storage.

Isolation of Native Rhizobia from Nodules

Air-dried nodules collected from MAC 13 and MAC 64 climbing beans were immersed in water and left to imbibe the water for 2 h. They were thoroughly washed and surface sterilized with 70% ethanol for 2 min and 3% sodium hypochlorite solution for 3 min (Somasegaran and Hoben, 1994). The sterile nodules were rinsed in seven changes of sterile distilled water and crushed in sterile universal bottles using a sterilized glass rod in normal saline solution. A loop-full of the resulting suspension was streaked on the surface of petri-dishes containing Yeast Extract Mannitol Agar (YEMA) medium supplemented with Congo red (0.0025% w/v). The streaked media were incubated in the dark at 28°C for 3–5 days (Vincent, 1970). The YEMA medium was prepared by dissolving 0.1 g NaCl; 10.0 g mannitol; 0.2 g MgSO₄·7H₂O; 0.5 g CaCO₃; 0.5 g yeast extract; 15.0 g agar and 0.002 M FeCl₂·6H₂O in 1 l of distilled water. Emerging single colonies, which were typical of rhizobia species, were sub-cultured by repeated streaking on YEMA and YEMA containing bromothymol blue (BTB) (0.0025% w/v) plates. Based on morpho-cultural and biochemical characteristics, the isolates were placed into nine different groups and a representative isolate from each group was used in symbiotic efficiency test. Pure cultures were preserved on YEMA agar slants in screw-capped McCartney bottles for future use at 4°C.

Greenhouse Experiments

The greenhouse experiments were carried out to assess infectivity and symbiotic efficiency of native rhizobia isolates on MAC 13 and MAC 64 climbing bean varieties under controlled conditions. The crops were grown in an Even-span greenhouse (polyethylene roofing) with the following conditions; natural lighting of 12-h day/night, temperatures of 20–25°C and relative humidity between 60 and 65%.

Authentication and Symbiotic Properties of the Native Rhizobia Isolates

Climbing bean seeds of high quality (undamaged) were surface sterilized for 30 s using 70% alcohol, followed by 3% sodium hypochlorite solution for 3 min and they were finally rinsed in seven changes of sterile distilled water. Twenty seeds were cultured in each petri-plate containing water agar (10% agar in distilled water) and pre-germinated aseptically in the dark at 28°C for 3 days. Only 2 actively sprouting seedlings with radicles of length 1–2 cm were transferred aseptically into each modified Leonard jars containing sterile vermiculite (rooting medium) and nitrogen-free nutrient solutions (Broughton and Dilworth, 1971) covered with sterile aluminum foil.

Inoculation of seedlings was carried out after 8 days by pipetting 1 ml (10^9 cfu/ml) of the respective rhizobia isolates into the root radical base. The greenhouse experiment was laid out in a completely randomized design (CRD) with 4 replicates per treatment. The treatments included; the representative native rhizobia isolates (ELM1, TUM2, ELM3, ELM4, ELM5, EUM6, EUM7, ELM8, and ELM9), native rhizobia consortium (TC), commercial rhizobia inoculant Biofix (TB) and a combination of all native rhizobia isolates + Biofix (TCB). Non-inoculated nitrogen treated (TN) and nitrogen-free (TUC) controls were included. The commercial rhizobia inoculant Biofix was supplied by MEA Company Limited, Nakuru, Kenya. The TN plants were constantly supplied with N (0.05% KNO_3 w/v) solution. The plants were grown for 45 days, regularly adding N-free nutrient solution as required. They were harvested for nodulation, dry weight biomass and shoot nitrogen and phosphorus determination. Shoot nitrogen was analyzed using Kjeldahl method (Bremner, 1996), while shoot phosphorus concentration was determined using the photometric method after sulfuric-perchloric acid digestion (Njeru et al., 2014). Symbiotic efficiency (SEF%) was determined as previously described by Gibson (1987) and Beck et al. (1993); $\text{SEF (\%)} = \frac{\text{Shoot dry weight (SDW) of inoculated plants}}{\text{SDW of non-inoculated control plants supplemented with nitrogen (0.05\% KNO}_3\text{)}} \times 100$ and then converted into a percentage. The SEF values were rated as: $>80\%$ = highly effective, $51\text{--}80\%$ = effective, $35\text{--}50\%$ = lowly effective and $<35\%$ = ineffective (Lalande et al., 1990).

Field Experiments

Field experiments were carried out to compare the effect of native rhizobia and commercial inoculants on nodulation, growth and seed yield of MAC 13 and MAC 64 climbing bean varieties. The experiments were laid out in a randomized complete block design (RCBD) with three replications. Treatments included; the best native rhizobia isolate (ELM3), a consortium (TC) of all native isolates (ELM1, TUM2, ELM3, ELM4, ELM5, EUM6, EUM7, ELM8, and ELM9), commercial inoculant Biofix (TB) and a mixture of a consortium of native isolates + Biofix (TCB). Non-inoculated control (TUC) was included as a negative control. The plant spacing used was 75 cm by 30 cm. Each plot measured 3×3 m and a spacing of 1 m between the plots was left to minimize inter-plot interference.

Land Preparation and Planting

The land was plowed and hand harrowed to a fine tilth before the first rain season (September 2015). A sterile filter mud was used as a carrier material for the rhizobia inoculants and inoculum was applied at recommended rate (100 g inoculum per 15 kg seeds) (Maingi et al., 2001). The Biofix commercial inoculum for beans was procured from MEA Company Limited and applied as per the manufacturer's instructions (100 g inoculum per 15 kg seeds). Only two climbing bean seeds of high quality were selected for planting. Seeds requiring rhizobia inoculation were prepared by coating with a filter mud containing respective inoculants using 4% gum Arabica (supplied with the inoculum). The negative control plots were left uninoculated and were planted a few hours before plots requiring inoculation in order to avoid cross

contamination. Each treatment received a basal application of $46 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ (TSP) fertilizers during planting.

Plant Growth and Harvesting

The climbing bean seedlings were thinned from two to one per hill 1 week after emergence. Weeds were controlled using hand hoeing over the growth period. During the mid-flowering stage, 3 plants from the central rows on each plot were selected randomly and harvested for assessment of nodulation and shoot biomass. The plant shoots were analyzed for nitrogen concentration (% N) using Kjeldahl method (Bremner, 1996), while shoot phosphorus was measured using photometry method after sulfuric-perchloric acid digestion (Njeru et al., 2014). At physiological maturity (after 95 days), 10 plants were randomly selected in each plot and manually harvested. Yield parameters per plant, such as the number of pods (NPP) and seed yield (SY) were assessed.

Data Analyses

The greenhouse and field data on nodule and shoot dry weight, shoot nitrogen and phosphorus, symbiotic efficiency, pod number and seed yield were subjected to analysis of variance (ANOVA) using Statistical Analysis Software (SAS) version 9.1. The means were separated using Tukey's HSD test at 5% significance level (Steel et al., 1997). Pearson correlation analysis was used to determine the association between nitrogen fixation parameters.

RESULTS

Soil Characteristics

The soil physical and chemical characteristics varied across the experimental sites in Embu and Tharaka Nithi Counties. The soils were characteristically acidic with pH ranging from 4.27 to 6.02 (Table 1). Soils from ELM had the highest organic carbon content (3.42%) and available phosphorus (32.15 ppm) while soil from EUM had the highest total nitrogen concentration (0.31%). The soil texture from EUM and TUM was classified as sandy clay, while soil from TLM and ELM were classified as clay and sandy clay loam, respectively (Table 1).

Morphological Characteristics of the Native Rhizobia Isolates

From this study, 9 distinct groups of isolates were obtained from the root nodules of MAC 13 and MAC 64 climbing beans grown during field trapping experiment in Eastern Kenya (Table 2). All isolates were identified as Gram-negative rods. The isolates did not absorb Congo red (CR) on streaking on YEMA-CR medium. There was no much variation observed in the colony shape, elevation and texture of the isolates. On YEMA-BTB medium, all isolates tested were acid producers and turned BTB indicator from deep green to yellow after 3 to 5 days of incubation in the dark. Out of the total 41 isolates obtained, group (iii) carried the highest percentage (28.89%), while group (ii) had the lowest percentage (2.22%) (Table 2).

TABLE 1 | Soil characteristics of experimental study sites (before planting) compared with the critical values for East African soils.

Soil sample	pH	% O.C	% Total N	K (cmol/kg)	Available P (ppm)	% Sand	% Clay	% Silt	Texture class
EUM	4.27	2.63	0.31	0.70	16.52	47	47	6	Sandy clay
ELM	6.02	3.42	0.22	0.85	32.15	54	27	19	Sandy clay loam
TUM	5.31	3.29	0.25	1.00	27.00	49	45	6	Sandy clay
TLM	5.85	3.33	0.20	1.80	25.10	45	49	6	Clay
*Critical value	5.50	3.00	0.25	0.22	15				

*Okalebo et al. (2002). ELM, Embu Lower Midland; EUM, Embu Upper Midland; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland; O.C, Organic Carbon; N, Nitrogen; K, Potassium; P, Phosphorus.

TABLE 2 | Morpho-cultural characteristics of the rhizobia isolates trapped from the study farms.

Characteristic	Isolate grouping								
	i	ii	iii	iv	v	vi	vii	viii	ix
CR absorption	Na	Na	Na	Na	Na	Na	Na	Na	Na
BTB reaction	Y	Y	Y	Y	Y	Y	Y	Y	Y
Gram reaction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Elevation	Cvx	Cvx	Cvx	Cvx	Cvx	Cvx	Cvx	Raised	Cvx
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Colony nature	Dull	Dull	Shiny	Shiny	Dull	Shiny	Shiny	Shiny	Shiny
Colony colour	Cy	Cw	Mw	Mw	Mw	Mw	W	Mw	Mw
Transparency	Op	Trl	Trl	Op	Op	Trl	Trl	Trl	Op
Colony dia. (mm)	1.5	3.5	1.0	3.0	0.5	3.5	5.0	1.0	1.0
Colony texture	Fg	Fg	Sm	Sm	Fg	Sm	Sm	Fg	Sm
Percentage %	4.44	2.22	28.89	6.67	8.89	20	17.78	4.44	6.67

Na, Non-absorbing; Y, Yellow on BTB; -ve, Negative; Cvx, Convex elevation; Ent, Entire margin; Shy, Shiny; Cy, Cream yellow; Cw, Cream white; Mw, milky white; W, watery; Op, Opaque; Trl, Translucent; Fg, Firm gummy; Sm, Soft mucoid; Diam, Colony diameter.

Symbiotic Efficiency of Native Rhizobia Isolates on MAC 13 and MAC 64 Climbing Beans

Upon inoculation of test plants with 9 representative native isolates in the greenhouse, the isolates ELM3, ELM4, ELM5, ELM8, and ELM9 initiated nodulation and were thus, considered for symbiotic efficiency (SEF) determination. Native isolates ELM1, TUM2, EUM6, and EUM7 were not considered for SEF determination because they did not form nodules with the climbing beans. There was a significant positive effect of the rhizobia isolates on NDW ($p < 0.001$), SDW ($p < 0.001$), and SEF ($p < 0.001$) (Table 3). A significant ($p < 0.001$) improvement of all the tested parameters was observed in climbing beans inoculated with native isolate ELM3 compared to the commercial inoculant Biofix and the negative control. The effect of bean variety on NDW was statistically significant ($p < 0.001$) with MAC 64 having higher NDW compared to MAC 13 climbing bean variety. On the contrary, there was no significant difference between the two varieties in regard to % shoot N ($p = 0.546$), shoot phosphorus ($p = 0.639$), and SEF ($p = 0.187$) (Table 3). SEF values ranged from 86.17% in TC to 123.72% in native isolate ELM3. There was no significant interaction effect between bean variety and rhizobia isolates on NDW ($p = 0.157$) and

SEF ($p = 0.885$). Correlation analysis showed a significant relationship between nodule dry weight and shoot dry weight of climbing beans ($R^2 = 0.5763$, $p = 0.029$; Figure 1). Similarly, there was a strong positive correlation between nodule dry weight and shoot nitrogen ($R^2 = 0.631$, $p = 0.021$; Figure 2).

Field Experiments

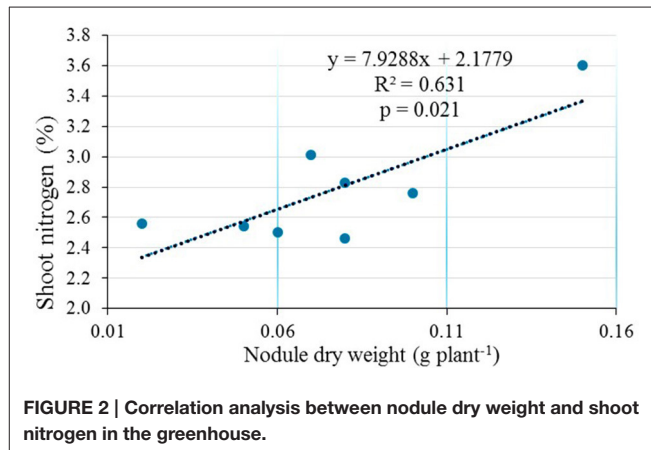
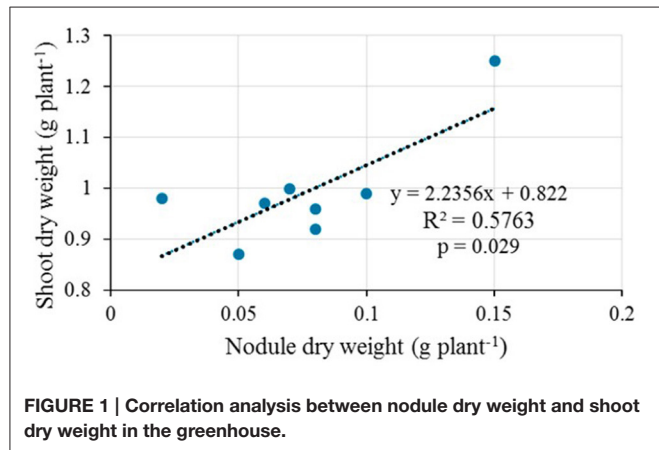
Effect of Rhizobia Inoculation on Nodulation and Plant Biomass

Rhizobia inoculation significantly enhanced NDW ($p < 0.001$) and SDW ($p < 0.001$) of climbing beans compared to non-inoculated controls (Table 4). Climbing beans inoculated with native isolate ELM3 had the highest NDW and SDW while non-inoculated beans had the lowest NDW and SDW (Table 4). The mean NDW and SDW of climbing beans inoculated with the native consortium, Biofix and a mixture of native consortium + Biofix were not statistically different. Between the two varieties, MAC 64 recorded a higher mean NDW of $0.09 \text{ g plant}^{-1}$ compared to MAC 13 with a mean of $0.08 \text{ g plant}^{-1}$. However, there was no significant ($p = 0.095$) difference in SDW of the two bean varieties. The climbing bean NDW and SDW varied significantly ($p < 0.001$) in all the farm sites with climbing beans from ELM recording the highest NDW and SDW while

TABLE 3 | Effect of rhizobia inoculation, bean variety, and their interaction on nodule dry weight, shoot dry weight, shoot nitrogen, and phosphorus and symbiotic efficiency in the greenhouse experiment.

Treatments	Nodule dry weight (NDW) g plant ⁻¹	Shoot dry weight (SDW) g plant ⁻¹	Shoot nitrogen (%N)	Shoot P (ppm)	Symbiotic efficiency (SEF) (%)
RHIZOBIA ISOLATES					
ELM3	0.147 ± 0.016a	1.25 ± 0.07a	3.46 ± 0.11ab	3543.75 ± 184.36ab	123.72 ± 6.72a
ELM4	0.015 ± 0.008cd	0.98 ± 0.09abc	2.56 ± 0.20cd	3109.38 ± 208.51bc	96.75 ± 8.67abc
ELM5	0.072 ± 0.019bc	1.00 ± 0.05abc	3.01 ± 0.08bc	3234.38 ± 431.97b	99.21 ± 4.97abc
ELM8	0.095 ± 0.020ab	0.99 ± 0.06abc	2.56 ± 0.09cd	3488.75 ± 513.93ab	98.24 ± 5.26abc
ELM9	0.083 ± 0.019b	0.92 ± 0.03bcd	2.46 ± 0.10cd	3979.88 ± 116.28a	90.76 ± 3.02bcd
TC	0.054 ± 0.016bcd	0.87 ± 0.06bcd	2.44 ± 0.12cd	2623.75 ± 200.32de	86.17 ± 5.67bcd
TB	0.077 ± 0.091b	0.96 ± 0.07abc	2.83 ± 0.07bc	3476.00 ± 308.06ab	95.21 ± 7.31abc
TN	-	1.01 ± 0.06ab	3.71 ± 0.09a	2436.63 ± 110.69e	100.00 ± 5.43ab
TCB	0.063 ± 0.015bc	0.97 ± 0.4abc	2.11 ± 0.14d	1771.88 ± 8.44f	96.53 ± 3.87abc
TUC	-	0.71 ± 0.03cd	0.73 ± 0.02e	3087.50 ± 394.18c	-
VARIETY					
MAC 13	0.035 ± 0.01b	0.96 ± 0.03a	2.37 ± 0.12a	3012.36 ± 159.67a	92.77 ± 2.43a
MAC 64	0.051 ± 0.01a	0.87 ± 0.03b	2.32 ± 0.11a	3046.21 ± 99.73a	88.59 ± 3.03a
P-VALUES OF THE MAIN FACTORS AND THEIR INTERACTIONS					
Rhizobia isolates	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Variety	0.0147	0.011	0.546	0.639	0.187
Variety × Rhizobia isolates	0.157	0.912	0.861	0.510	0.885

Means followed by same lower case letter(s) within the same column are not significantly different at $p < 0.05$ according to Tukey's HSD test. ELM3, ELM4, ELM5, ELM8 and ELM9, Native rhizobia isolates; TC, Consortium of native rhizobia; TB, Commercial inoculant (Biofix); TN, Negative control with nitrogen treatment; TCB, Biofix combined with consortium; TUC, Negative control without nitrogen; MAC, Mid altitude climbers. ELM, Embu Lower Midland.



climbing beans grown in EUM recorded the lowest NDW and SDW (Table 4). There was no significant ($p < 0.052$) interaction effect of rhizobia inoculation × site observed on bean nodulation.

Effect of Rhizobia Inoculation on Shoot Nitrogen (% N) and Phosphorus (P)

The level of shoot nitrogen (% N) and phosphorus concentration were significantly ($p < 0.001$) enhanced by rhizobia inoculation (Table 4). Climbing beans inoculated with native isolate ELM3, had the highest shoot % N and P, while non-inoculated controls had the lowest % N and P (Table 4). Unlike in the greenhouse,

there was a significant variation in % N and P ($p = 0.001$, $p < 0.001$, respectively) accumulated by the two climbing bean varieties, where MAC 64 recorded a higher shoot % N and P compared to MAC 13. Farm site significantly influenced the mean % shoot N concentration ($p < 0.001$) and shoot phosphorus ($p = 0.021$). Among the four sites, climbing beans grown in Embu County (ELM and EUM) recorded higher mean % N compared to those in Tharaka Nithi County (TLM and TUM). Interestingly, climbing beans in EUM accumulated the highest amount of P (9,335.8 ppm) despite performing poorly in nodulation. Additionally, there was a significant ($p = 0.010$)

TABLE 4 | Effect of rhizobia inoculation, bean variety, farm site, and their interactions on nodule dry weight, shoot dry weight, shoot nitrogen and phosphorus in the field.

Treatments	Nodule dry weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	% Shoot N	Shoot P (ppm)
RHIZOBIA INOCULANT				
ELM3	0.11 ± 0.06a	11.90 ± 0.80a	3.342 ± 0.115a	3608.60 ± 192.49a
TC	0.08 ± 0.02b	9.58 ± 0.76b	2.179 ± 0.099d	2609.20 ± 256.97c
TB	0.09 ± 0.01b	10.56 ± 0.73b	3.051 ± 0.081b	3268.80 ± 193.23ab
TCB	0.09 ± 0.02b	9.92 ± 0.78b	2.579 ± 0.057c	3120.00 ± 202.54b
TUC	0.06 ± 0.01c	7.34 ± 0.54c	1.766 ± 0.134e	3018.10 ± 241.10b
VARIETY				
MAC 13	0.08 ± 0.01a	9.63 ± 0.49a	2.499 ± 0.105b	2721.30 ± 116.85b
MAC 64	0.09 ± 0.01b	10.08 ± 0.49a	2.667 ± 0.089a	3528.62 ± 147.61a
SITE				
EUM	0.05 ± 0.01c	4.79 ± 0.23c	2.790 ± 1.01a	3335.80 ± 205.31a
ELM	0.11 ± 0.01a	13.66 ± 0.49a	2.844 ± 1.49a	2956.30 ± 210.85b
TUM	0.09 ± 0.02b	10.87 ± 0.35b	2.280 ± 0.93b	3105.70 ± 228.51ab
TLM	0.09 ± 0.01b	10.13 ± 0.38b	2.419 ± 1.06b	3102.10 ± 159.10ab
P-VALUES OF THE MAIN FACTORS AND THEIR INTERACTIONS				
Variety	0.015	0.095	0.001	< 0.001
Site	< 0.001	< 0.001	< 0.001	0.021
Rhizobia inoculant	< 0.001	< 0.001	< 0.001	< 0.001
Variety × Site	0.069	0.993	0.052	0.061
Variety × Rhizobia inoculant	0.498	0.967	0.719	0.071
Site × Rhizobia inoculant	0.052	0.217	0.010	0.051
Site × Rhizobia inoculant × Variety	0.414	0.827	0.064	0.102

Means followed by same lower case letter(s) within the same column are not significantly different at $p < 0.05$ according to Tukey's HSD test. ELM3, Test native rhizobia isolate; TC, Consortium of native rhizobia; TB, Commercial inoculant (Biofix); TCB, Biofix combined with consortium; TUC, Negative control (Non-inoculated); MAC, Mid altitude climbers. ELM, Embu Lower Midland; EUM, Embu Upper Midland; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland.

interaction effect between farm site and rhizobia inoculants on climbing bean shoot % N (Table 4). In this case, climbing beans inoculated with native isolate ELM3 accumulated a higher % shoot N in all the sites (Figure 3).

Effect of Rhizobia Inoculation on Pod Number (NPP) and Seed Yield (SY)

Rhizobia inoculation significantly enhanced ($p < 0.001$) pod number (Figure 4A) and seed yield (Figure 4B), whereby inoculated climbing beans recorded higher NPP and SY values compared to non-inoculated control. Climbing beans inoculated with native isolate ELM3 produced the highest NPP (29.75 plant⁻¹) and SY (4,397.75 kg ha⁻¹), while non-inoculated plants recorded the lowest NPP (16.44 plant⁻¹) and SY (2,324.81 kg ha⁻¹). The enhanced SY by isolate ELM3 represents 89% increase over the non-inoculated controls and 30% increase over the commercial inoculant Biofix (3,698.79 kg ha⁻¹). Similarly, farm site significantly affected ($p < 0.001$) the NPP (Figure 4C) and SY (Figure 4D). Climbing beans grown in TLM and EUM agroecological zones produced the least SY. Correspondingly, bean variety significantly affected NPP ($p = 0.001$) and SY ($p = 0.002$) with MAC 64 having a higher NPP (Figure 4E) and SY (Figure 4F) compared to MAC 13 bean variety. A significant ($p = 0.001$) interaction effect was observed on SY between farm site and bean

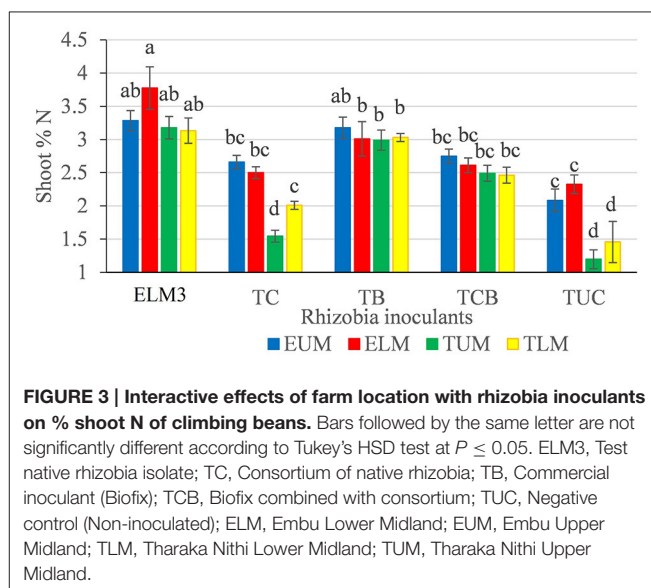


FIGURE 3 | Interactive effects of farm location with rhizobia inoculants on % shoot N of climbing beans. Bars followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. ELM3, Test native rhizobia isolate; TC, Consortium of native rhizobia; TB, Commercial inoculant (Biofix); TCB, Biofix combined with consortium; TUC, Negative control (Non-inoculated); ELM, Embu Lower Midland; EUM, Embu Upper Midland; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland.

variety. In this case, MAC 64 bean variety recorded the highest seed yield (4,691.26 kg ha⁻¹) in ELM while MAC 13 produced the lowest seed yield (2,644.05 kg ha⁻¹) in EUM (Figure 5).

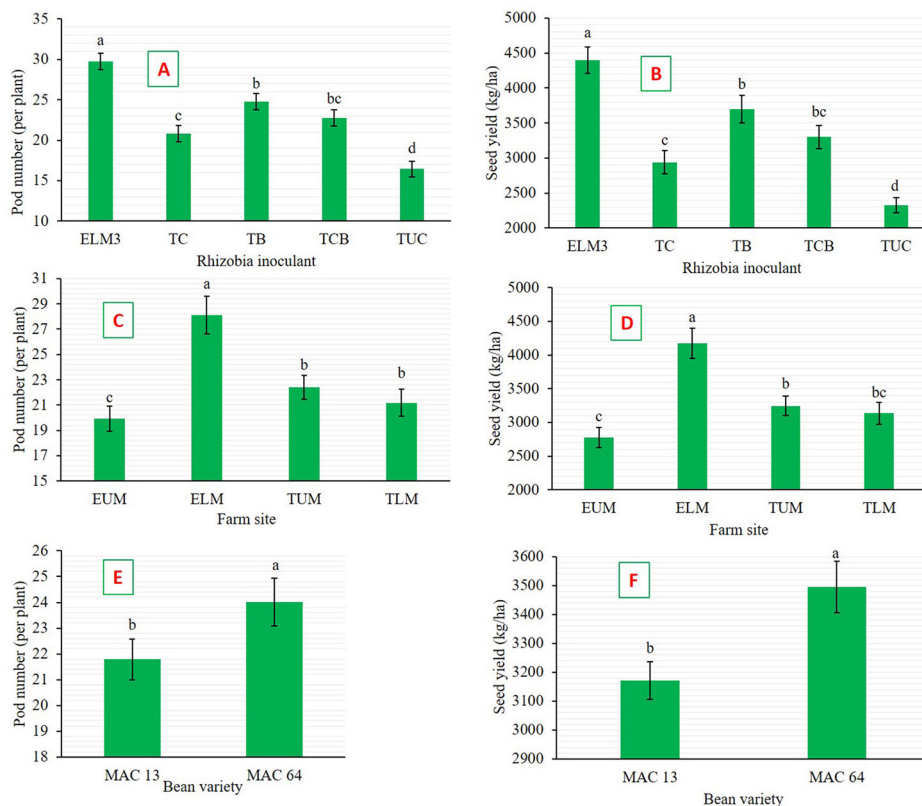


FIGURE 4 | Effects of the main factors on climbing bean yield parameters in the field. (A) Effect of rhizobia inoculation on pod number. **(B)** Effect of rhizobia inoculation on seed yield. **(C)** Effect of farm site on pod number. **(D)** Effect of farm site on seed yield. **(E)** Effect of bean variety on pod number. **(F)** Effect of bean variety on seed yield. Bars followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. ELM3, Test native rhizobia isolate; TC, Consortium of native rhizobia; TB, Commercial inoculant (Biofix); TCB, Biofix combined with consortium; TUC, Negative control (Non-inoculated); ELM, Embu Lower Midland; EUM, Embu Upper Midland; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland.

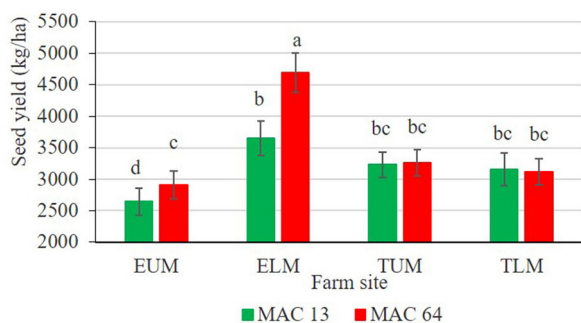


FIGURE 5 | Interactive effects of bean variety with farm site on seed yield of climbing beans. Bars followed by the same letter are not significantly different according to Tukey's HSD test at $P \leq 0.05$. ELM, Embu Lower Midland; EUM, Embu Upper Midland; TLM, Tharaka Nithi Lower Midland; TUM, Tharaka Nithi Upper Midland.

DISCUSSION

In the present study, the pH of soil across the farms varied in respect to the agro-ecological zonation. This could be attributed

to the high mineralization rates and loss of exchangeable bases (Ca, K, and Mg) that occur through leaching in upper slopes of Mt. Kenya region (Mairura et al., 2008; Mwenda et al., 2011). Climbing beans require slightly acidic or neutral soil for growth especially when the crops depend on BNF as a source of nitrogen (Martínez-Romero, 2003). Soil pH below 5.5 and high nitrogen values above the critical limit of 0.25% described by Okalebo et al. (2002), suppresses nodulation and nitrogen fixation and does not favor the production of climbing beans (Mairura et al., 2008). The sandy-clay-loam soil texture that was recorded in ELM farm promotes soil drainage and infiltration; giving favorable conditions for bean production. A well-drained, deep, light textured soil with adequate porosity is ideal for survival and proliferation of soil bacteria, such as root-nodule rhizobia living within the plant rhizosphere (Katungi et al., 2011).

The morpho-cultural characteristics of the isolates based on Gram staining results and growth on YEMA-CR and YEMA-BTB media under dark incubation, confirmed the standard morpho-cultural characteristics of *Rhizobium* species as described by Vincent (1970), Beck et al. (1993), and Somasegaran and Hoben (1994). Morphological characteristics of rhizobia which nodulated climbing beans in the present study are similar to

those reported by Kawaka et al. (2014) and Muthini et al. (2014). Temperature, pH, and soil salinity are among the abiotic factors that affect morphological characteristics of rhizobia. The isolates exhibiting a wide adaptation to environmental stresses, and are able to circumvent limiting factors and maintain a higher capacity for nitrogen fixation, could be considered for inoculum development (Berrada et al., 2012).

The greenhouse results showed that rhizobia inoculation significantly enhanced climbing bean nodulation, dry weight biomass, and symbiotic efficiency. Some of the native isolates, such as ELM3 showed superior performance in all the tested symbiotic parameters compared to the commercial inoculant Biofix and non-inoculated control. The enhanced performance showed by some of the native isolates could be attributed to their ability to infect, nodulate and fix nitrogen with MAC 13 and MAC 64 climbing beans. On the other hand, the performance of commercial inoculant is largely dependent on not only the number of viable rhizobia cells but also the presence or absence of other microbial contaminants (Aliyu et al., 2013). These findings are consistent with the previous findings (Onyango et al., 2015), which showed the competitive potential of Bambara native isolates from Western Kenya when compared to the commercial strain USDA 110. Most of the root nodules from our study showed pink coloration, indicating the presence of iron-containing protein required for effective nitrogen fixation (Farid and Navabi, 2015). Pink nodules are known to contain and actively express *nifH* genes that codes for the synthesis of nitrogenase enzymes responsible for the reduction of N to NH_3 (Rondon et al., 2007). Between the two climbing bean varieties, MAC 64 showed superior nodulation and biomass accumulation over MAC 13 under controlled greenhouse conditions. The high nodule dry matter recorded by MAC 64 reflects a more efficient symbiotic nitrogen fixation that could result to an increased shoot biomass. These results are consistent with the observations made by Gicharu et al. (2013), who noted the differences in nodulation and biomass accumulation among three climbing bean cultivars (G59/1-2, NG224-4, and Cargamanto) grown under controlled conditions in the greenhouse. Our findings may, therefore, suggest the superior performance of MAC 64 over MAC 13 climbing bean varieties in the greenhouse environment.

The plant SDW was used during the study to estimate the symbiotic nitrogen-fixing efficiency of the native rhizobia isolates (Gibson, 1987; Beck et al., 1993). This method is easy to use and it is relatively cheap; and most appropriate for use in soils with low nitrogen content (Rondon et al., 2007). Symbiotic efficiency (SEF) differed significantly among the isolates tested in the greenhouse. In our study, all the native isolates that nodulated had higher SEF, which ranged from 86.7 to 123.72%. Such findings suggest that native isolates enhanced nitrogen fixation, which consequently increased SDW and nitrogen accumulation. These results concur with the findings of Kawaka et al. (2014) who reported SEF ranging between 67 and 170% when common beans were inoculated with native rhizobia in Western Kenya. Similarly, Mungai and Karubiu (2011) reported that native rhizobia isolated from common beans from Njoro, Kenya, had higher SEF compared to the commonly used commercial inoculants Biofix and USDA 9030. Based on the rating scale used by Lalonde et al. (1990), it was evident from our study that native

isolates ELM3, ELM4, ELM5, ELM8, and ELM9 were highly effective (SEF > 80%) in symbiotic nitrogen fixing efficiencies. The consortium of native isolates had the lowest SEF, inferring that the combination of several native isolates could not provide any functional advantage compared to single isolates (Meghvansi et al., 2010).

The significant correlation between nodule dry weight and shoot dry weight in the greenhouse experiments confirmed the dependence of shoot biomass on nodulation (Kawaka et al., 2014). In addition, the results from this study support the assertion made by DeliĆ et al. (2010), that there is a direct relationship between nodulation and nitrogen accumulation in legumes. Our findings, therefore, demonstrated that rhizobia inoculation enhanced nodulation and nitrogen fixation, which improved shoot nitrogen nutrition and plant biomass. These results concur with the findings of Unkovich et al. (2010) who reported a strong positive correlation between shoot biomass and nitrogen accumulated by rhizobia-inoculated lentils and peas. This study supports the use of such parameters as measures of nitrogen fixation potential of rhizobia isolates in symbiosis with legumes (Patra et al., 2012).

Inoculation of climbing beans in the field significantly enhanced nodule and shoot dry weights, pod number and seed yields of MAC 13 and MAC 64 climbing beans. In our study, the superiority of native isolate ELM3 over other inoculants, in respect to nodulation, biomass accumulation and yield, indicate the existence of effective rhizobia isolates in the soil of Eastern Kenya. Native strains are more competitive in nodule infection and occupancy compared to commercial inoculants because they are well adapted to the local agro-climatic conditions (Meghvansi et al., 2010). In addition, native rhizobia strains interact positively with the resident microbial populations resulting to improved soil health, nutrient availability and enhanced yields (Nkot et al., 2015; Tena et al., 2016). Rhizobia interact positively with other microbial inoculants, such as arbuscular mycorrhizal fungi resulting to improved soil health, nutrient availability and enhanced crop yields (Meng et al., 2015; Oruru and Njeru, 2016), therefore showing its importance in sustainable agricultural farming practices.

According to Morad et al. (2013), inoculated beans showed higher nodulation, pod number and seed yield compared to the control plants, which was in line with the findings of our study. Interestingly, the individual performance of native isolates in the field was better than that of the commercial inoculants. Our findings are further supported by Tena et al. (2016) who concluded that native rhizobia isolates in the field often out-compete the commercial rhizobia inoculant, highlighting the potential of native isolates in bean production. Nonetheless, commercial rhizobial inoculants have potential to enhance growth and nitrogen fixation of legumes (Thuita et al., 2011; Ulzen et al., 2016). In this study, the average performance of Biofix in nitrogen fixation could be attributed to the soil properties and unfavorable agroecological conditions in Eastern Kenya, which affects the rhizobia-legume interaction within the rhizosphere. Meghvansi et al. (2010) further stress the importance of using effective native rhizobia, which are adapted to the local soil and environmental conditions as biofertilizer inoculants in bean production.

Inoculation of climbing beans with native consortium isolates and the combination of native consortium + Biofix had no significant effect on NDW and SDW. This shows that diversifying rhizobia isolates in our field study had no beneficial advantage over the use of single rhizobia isolates. Martínez-Romero (2003) noted that bean inoculation with a diverse rhizobia population does not necessarily translate to a higher legume grain yield due to the inability of some rhizobia strains to cause nodulation and affect nitrogen fixation. In addition, Nkot et al. (2015) added that the establishment, persistence, and effectiveness of an introduced rhizobia strain often decrease with increase in population density due to the possibility of negative microbial interaction or incompatibility with the other symbionts within the rhizosphere.

In this study, there was a high percentage shoot N and P recorded in climbing beans inoculated with native isolate ELM3 in all the four agroecological sites. This demonstrated the consistency and superiority of the native isolate in nitrogen fixation both in the greenhouse and in the field conditions. Phosphorus is an essential nutrient that drives BNF, therefore, P-deficient soils could result into low BNF despite the high abundance of native rhizobia strains (Unkovich et al., 2010). In our study, phosphorus was applied in form of (TSP) fertilizers at a rate of 46 kg P₂O₅ ha⁻¹ to achieve maximum nitrogen fixation (Gicharu et al., 2013). The significant interactive effects between bean variety and farm site on seed yield indicate that the two bean varieties responded differently in the four agroecological zones of Eastern Kenya. The MAC 64 climbing beans grown in Embu lower midland agroecological zone produced the highest climbing bean seed yield. This could be attributed to various factors including favorable soil pH, the soil type, effectiveness of rhizobia and climatic conditions (Rondon et al., 2007). Monyo and Laxmipathi (2014) reported that different soybean and common bean varieties are suitable for production in specific agroecological zones of Malawi due to the varying ecological and climatic conditions.

This study has proved the superiority of native rhizobia over introduced inoculants in four different agroecological zones with no history of previous inoculation. Thus, the study recognizes the sense of the “geographical place factor” for microorganisms to adapt to different physical and biological properties of an agricultural ecosystem. The use of native rhizobia in supplementing nitrogen-based chemical fertilizers in legume production is of great significance as it promotes local self-management of natural agroecosystems for legume production (Meghvansi et al., 2010). According to Nkot et al. (2015), the use of native rhizobia as bio-fertilizers would enhance soil biodiversity conservation, because bio-fertilization limits the adverse negative effects brought about by the inorganic fertilizers on below-ground biodiversity. The effective use of locally available rhizobia strains, therefore, promotes the delivery

of sustainable agroecosystem services (Singh et al., 2016) in legume production.

CONCLUSION

Generally, the current study demonstrated the presence of effective native rhizobia that are potentially superior compared to the available commercial inoculant (Biofix) in nodulation, symbiotic efficiency and yield performance of MAC 13 and MAC 64 climbing bean varieties. There exists a significant variation in symbiotic efficiencies of native rhizobia isolates nodulating with MAC 13 and MAC 64 climbing beans. The native isolate ELM3 had the highest symbiotic efficiency compared to other isolates tested in the greenhouse. Consistencies in the performance isolate ELM3 in the greenhouse as well as in all the four agroecological zones of Eastern Kenya clearly indicate its symbiotic superiority and adaptability to the region. The low efficiency of the combination of Biofix + a consortium of native isolates indicates that diversifying rhizobia isolates in the field had no additive effect over the use of independent isolates. Based on the superior response of MAC 64 climbing bean variety toward rhizobia inoculation, as evident in the greenhouse and field experiments, further breeding of this variety should be considered. Moreover, farmers should adopt the use of effective native rhizobia inoculants to enhance climbing bean production in the region. Further experiments should focus on genetic characterization and large-scale multiplication of the effective native isolate ELM3 before being recommended as a bean rhizobia inoculant.

AUTHOR CONTRIBUTIONS

JK, OO, JM, and EN conceived and designed the research and data collection tools and participated in drafting the manuscript. GK and SM collected the data, participated in data analyses and wrote the manuscript. EN performed data analyses. All authors read and approved the final manuscript.

FUNDING

This work was supported by the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) 5th Graduate Research Grant (GRG), Grant No. RU 2014 GRG-102.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to RUFORUM for supporting this study and participating households from Embu and Tharaka Nithi Counties of Eastern Kenya for their cooperation, participation, patience and time during the collection of the data.

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Conflict of Interest Statement: 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.

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Regional Conditions and Land-Use Alter the Potential Contribution of Soil Arthropods to Ecosystem Services in Grasslands

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OPEN ACCESS

Edited by:

Alberto Orgiazzi,
European Commission, Joint
Research Centre, Italy

Reviewed by:

Aidan M. Keith,
Centre for Ecology & Hydrology, UK
Roel Van Klink,
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Ecology and Evolution

Received: 30 October 2015

Accepted: 14 December 2015

Published: 19 January 2016

Citation:

Birkhofer K, Dietrich C, John K,
Schorpp Q, Zaitsev AS and Wolters V
(2016) Regional Conditions and
Land-Use Alter the Potential
Contribution of Soil Arthropods to
Ecosystem Services in Grasslands.
Front. Ecol. Evol. 3:150.
doi: 10.3389/fevo.2015.00150

We investigated the impact of regional conditions and land-use intensity on eight selected arthropod taxa of Mesostigmata (Parasitidae), Oribatida (three species), Collembola (one species), Chilopoda (two species), and Diplopoda (one species) sampled in differently managed permanent grasslands of three German study regions. By jointly analyzing changes in abundance and trophic behavior (measured as natural variation in $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios) we intended to develop a framework for evaluating the impact of local and regional conditions on the ecosystem services delivered by soil animals (mainly decomposition- and predation-related services). The investigated taxa could be assorted to three major groups: (1) numerical response only, (2) numerical and trophic response and (3) trophic response only. Since the combination of taxa assembled in the individual groups does not correspond to any of the conventional soil ecological classification systems, this grouping offers a new approach for analyzing soil communities. The complementing consideration of both the direction of the numerical response and the type of the trophic response (change of the basal food source vs. trophic level shift vs. variations in isotopic niches) provided a differential insight into the effect of management and geographic differences on soil arthropods. It could be shown that the effect of land-use on the abundance of detritivorous microarthropods varies among regions, but does not induce any changes in feeding behavior. Our findings on Parasitidae indicate that carnivorous microarthropods exert substantial predation pressure on soil mesofauna and may be quite resistant to environmental changes due to high trophic flexibility. If conditions are favorable, centipedes may reach comparatively high densities in permanent grasslands and could be very important for controlling belowground pests. Concerning millipedes, isotopic signatures suggest that some species could exert a substantial disservice by feeding on roots over a wide range of land-use intensities and regional conditions. We conclude that the many consistent and significant effects found in our study support our contention that the combined analysis of numerical and trophic responses provides a promising framework for designing spatially explicit models that quantify the impact of human interventions on the delivery of ecosystem services by the soil fauna.

Keywords: soil fauna, ecosystem services, permanent grasslands, land-use intensity, stable isotopes, trophic response

INTRODUCTION

Edaphic animals affect a wide range of ecological processes and soil-related ecosystem services (Brussaard, 1997; Heemsbergen et al., 2004; de Vries et al., 2013) with detritivores *inter alia* contributing to plant nutrition, carbon turnover, detoxification, and soil quality (Wardle et al., 2004; Nielsen et al., 2011). Moreover, soil predators are important natural regulators of belowground pests (Zenger and Gibb, 2001). However, spatially explicit predictions of such services and their magnitude are difficult, since the contribution of soil animals to ecosystem processes may considerably differ among sites due to local or regional differences in the abundance, the functional role or both of the species involved. The significant impact of regional conditions on the composition of edaphic animal communities is well established (Fierer et al., 2009; Birkhofer et al., 2012; Tsiafouli et al., 2015). Similarly, strong alterations of soil animal assemblages by land-use have often been reported for the local scale (e.g., Birkhofer et al., 2008). The interplay between regional conditions and local land-use is therefore likely to modify the contribution of edaphic animals to ecosystem services. It remains unknown, however, to what extent such changes manifest themselves through numerical and/or functional responses of the soil fauna to large- and small-scale differences in environmental conditions (Barrios, 2007; Crotty et al., 2014).

The study presented here focusses on edaphic arthropods of grasslands. Grassland soils host diverse invertebrate communities that are known to sensitively respond to management (e.g., Curry, 1994; Dahms et al., 2010; Menta et al., 2011). This, for example, has been demonstrated for the effects of fertilization (King and Hutchinson, 1980; Birkhofer et al., 2008), grazing (King and Hutchinson, 1976; Ponge et al., 2015), and cutting (Jensen et al., 1973; Lemanski and Scheu, 2015). As a consequence, critical shifts within soil food webs may occur (Haubert et al., 2009; Birkhofer et al., 2011; Crotty et al., 2014; Klarner et al., 2014). Unfortunately, the greening measures recently implemented by the European Commission to protect and maintain species-rich permanent grasslands do not take such human interventions into account (Common Agricultural Policy 2013: Regulation No 1307/2013 Article 4).

Most studies on the arthropods of grassland soils quantify changes in abundance patterns, while functional responses are rarely addressed. Stable isotope analysis provides a unique opportunity for overcoming this gap of knowledge (e.g., Scheu and Falca, 2000; Birkhofer et al., 2011). Concerning soil communities, this technique has been applied to determine trophic niches (Schneider et al., 2004; Chahartaghi et al., 2005), food web structure (Erdmann et al., 2007; Pollierer et al., 2009; Klarner et al., 2013), and patterns of basal resource utilization (Albers et al., 2006; Sereda et al., 2015). The $^{15}\text{N}/^{14}\text{N}$ ratio can be used to identify the trophic position of species in soil food webs (DeNiro and Epstein, 1981), since the heavy nitrogen isotope ^{15}N is usually enriched in consumers compared to their food source. The $^{13}\text{C}/^{12}\text{C}$ ratio on the contrary allows for estimating the basal food resource of a consumer as it is usually only enriched by about 0.4‰ compared to the food source (Post, 2002). The joint analysis of these two isotope ratios therefore provides a versatile

tool to measure management induced shifts in the provision of those ecosystem functions and services that depend on the trophic structure of belowground biota (Klaus et al., 2013).

Here we aim at developing a framework for evaluating the joint impact of regional conditions and land-use intensity on the abundance and functional role of edaphic animals (with function being measured as natural variation in $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios). We consider such a framework as an essential prerequisite for spatially explicit predictions of decomposition- and predation-related services provided by the soil fauna. The study focusses on eight selected arthropod taxa sampled in differently managed permanent grasslands of three German study regions (cf. Fischer et al., 2010). These taxa represent a wide range of soil taxonomic and functional groups.

MATERIALS AND METHODS

Study Sites and Land-Use Intensity

The grassland sites were located in three regions of Germany that span a latitudinal gradient of more than 500 km (DFG Biodiversity-Exploratories, Fischer et al., 2010). The northernmost region Schorfheide-Chorin (SEG) is situated in the lowlands of north-eastern Germany and soils are dominated by glacially formed, sandy bog soils. It has an approximate size of 1300 km². The region Hainich-Dün (HEG) is located in central Germany and soils contain more clay and form stagnosols with poor water penetration. It covers an area of ~1300 km². The southernmost region Schwäbische Alb (AEG) is situated in the low mountain ranges of south-western Germany and soils are dominated by limestone derived Rendzina. The approximate size of this region is 422 km². Grasslands included meadows, pastures, and mown pastures in each study region and all plots have not been part of a crop rotation scheme for at least 8 years. Land-use intensity was characterized by a land-use index (LUI, Blüthgen et al., 2012) that has been particularly developed for these grassland sites in the biodiversity exploratory regions. It jointly incorporates information about grazing intensity, cutting frequency, and fertilization intensity from 2008 to 2010 (i.e., average of the 3 year preceding our sampling in spring 2011).

Arthropod and Plant Sampling

All 150 grasslands of the Biodiversity-Exploratories (50 plots per region) were sampled between April 11 and April 21 in spring 2011. At each grassland site, a 2 × 2 m subplot was established with a minimum distance of 20 m to field edges. Two soil cores with a diameter of 20 cm, a depth of 10 cm, and a distance of 1 m from each other were taken in random locations in these subplots. The vegetation was removed from the top of one soil core and the upper 5 cm were stored under cool conditions until soil animal extraction via a modified Kempson method commenced (Kempson et al., 1963). The second soil core was cut to a depth of 10 cm and immediately hand sorted for soil macrofauna (centipedes and millipedes) for 1 h per core. The soil fauna from both soil cores was transferred to 70% Ethanol for later identification. Though ethanol like other preservatives (e.g., formalin) can affect stable isotope values (Carabel et al., 2009),

we do not consider this to cause a serious bias since all samples were stored and treated identically. Three randomly located samples of the growing vegetation were pooled in each subplot and processed for the plot-specific estimate of isotope ratios in basal resources. Further analyses on soil invertebrates were confined to eight arthropod taxa that occurred in all three study regions (listed in Table 1). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

Stable Isotope Analysis

Soil arthropods and plant material were dried at 60°C for 24 h prior to stable isotope analyses and material for each sample was then homogenized with a ball mill. Depending on the body size of the respective species between 1 and 50 individuals were pooled. Between 0.25 and 0.69 mg of soil arthropods per species and 3.0–4.0 mg of plant material were processed for each stable isotope sample. Living plants and their debris often have fairly similar isotope ratios. This becomes obvious when comparing the results of Klaus et al. (2013) for grassland litter to those of Kleinebecker et al. (2014) for foliar material (both studies where carried out in the Biodiversity-Exploratories). Therefore, isotope values of the standing biomass were assumed to reflect isotope values of the litter available for the decomposer food web. Isotope ratios in animal and plant samples as well as reference materials were measured using a coupled system of an elemental analyzer and isotope ratio mass spectrometer (EA-IRMS). The reference used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis for vegetation samples was IA-R001 (wheat flour, $\delta^{13}\text{C-V-PDB} = -26.43\text{‰}$, $\delta^{15}\text{N-Air} =$

2.55 ‰). The reference used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis of soil arthropod samples was IA-R042 (powdered bovine liver, $\delta^{13}\text{C-V-PDB} = -21.60\text{‰}$, $\delta^{15}\text{N-Air} = 7.65\text{‰}$). IA-R001 and IA-R042 are calibrated against and traceable to IAEA-CH-6 (sucrose, $\delta^{13}\text{C-V-PDB} = -10.43\text{‰}$) and IAEA-N-1 (ammonium sulfate, $\delta^{15}\text{N-Air} = 0.40\text{‰}$). Isotopic signatures are expressed using the δ notation with $\delta X (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1000$, with X representing the target isotope and R_{sample} is the $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{standard} the respective ratio of the standard (Peterson and Fry, 1987).

Stable isotope measurements need calibration prior to comparison of signatures of soil animal species between regions. Physiological traits of plants in different regions (Chevillat et al., 2005; Kuptz et al., 2011) and regional differences in nitrogen deposition, precipitation, mean temperature (Boeckx et al., 2005), or land-use history (Kleinebecker et al., 2014) may lead to systematic differences between study regions. Studies in forest ecosystems have shown that both leaf litter and fine roots are suitable basal resources to calibrate signatures of animal species (Klarner et al., 2014). In grassland ecosystems, plant litter is not very abundant, as the limited amount of grass or herb litter is rapidly incorporated into the soil by macrodecomposers (e.g., earthworms). We therefore calibrated isotope values for soil arthropods as difference between the aboveground plant and soil arthropod samples ($\delta_{\text{plant}} - \delta_{\text{animal}}$) at a particular site (expressed by the notion $\Delta^{15}\text{N}$ or $\Delta^{13}\text{C}$). The resulting enrichment values for nitrogen and carbon isotopes suggest that values are comparable to calibration by litter samples (Crotty et al., 2014; Mesostigmata N: +6‰, C: +3‰;

TABLE 1 | Soil arthropod taxa occurring in the grasslands of all three regions [SEG, Schorfheide-Chorin (northernmost); HEG, Hainich-Dün (central); AEG, Schwäbische Alb (southernmost)].

Species/Family	Taxonomic group	Trophic level	R	Sites and abundances					
				SEG	n	HEG	n	AEG	n
MESOFAUNA									
Parasitidae	Acari: Mesostigmata	P	0.60	14	179.3 ± 39.1	14	117.4 ± 19.5	15	207.9 ± 38.5
<i>Eupelops occultus</i> (Koch, 1835)	Acari: Oribatida	D	0.48	8	168.2 ± 55.7	3	54.0 ± 15.3	14	192.0 ± 42.0
<i>Tectocephus velatus</i> (Michael, 1880)	Acari: Oribatida	D	0.35	3	168.2 ± 89.9	6	334.9 ± 106.2	2	134.9 ± 36.2
<i>Trichoribates trimaculatus</i> (Koch, 1836)	Acari: Oribatida	D	0.91	11	114.3 ± 36.6	2	7.9 ± 3.9	7	65.1 ± 25.0
<i>Isotoma viridis</i> (Bourlet, 1839)	Collembola: Entomobryoida	D	0.69	4	122.2 ± 52.1	4	52.4 ± 17.5	3	51.7 ± 15.8
MACROFAUNA									
<i>Geophilus flavus</i> (De Geer, 1778)	Chilopoda: Geophilomorpha	P	0.66	5	5.0 ± 2.3	27	38.7 ± 7.6	19	20.7 ± 4.9
<i>Schendyla nemorensis</i> (Koch, 1835)	Chilopoda: Geophilomorpha	P	0.73	4	5.3 ± 2.6	21	47.7 ± 10.5	17	25.7 ± 6.8
<i>Cylindroiulus caeruleocinctus</i> (Wood, 1864)	Diplopoda: Julida	D	0.74	11	8.7 ± 3.1	26	16.7 ± 3.2	14	7.7 ± 2.4

Listed are the major taxonomic groups, the trophic levels (D, decomposer; P, predator), the Pearson correlation coefficient between $\delta^{15}\text{N}$ values in animal samples and plant samples (R), the number of sites within each region for which abundances were sufficiently high to analyze ratios of naturally occurring C and N stable isotopes and the mean abundances of each taxonomic group per region (n, individuals m⁻²) ± standard errors of the mean for each region and taxon.

Oribatida N: +2‰, C: +2‰, and Entomobryid Collembola N: +2‰, C: 0‰). Our approach is further supported by strong correlations between $\delta^{15}\text{N}$ ratios in local plant samples and soil arthropod values in the respective grassland plots, with correlation coefficients ranging from 0.4 to 0.9 for the eight taxa analyzed (Table 1).

Statistical Analysis

Isotope values of soil arthropod species and plant samples were analyzed with permutational analysis of variance by permutation of residuals under a reduced model (PERMANOVA, Anderson, 2001) with the fixed factor “Region” (levels Schorfheide-Chorin, Hainich-Dün or Schwäbische Alb) and the continuous predictor “Land-use intensity” (LUI). All PERMANOVA analyses were based on Euclidean distances and 9999 permutations and statistical tests were performed in the software Primer-E (Clarke and Gorley, 2006) with the Permanova+ add-on (Anderson et al., 2008). Abundances of soil arthropod species were analyzed with a generalized linear model for count data (Poisson model with log link) with the same model terms as in the PERMANOVA. These analyses were performed in Statistica 12 (StatSoft Inc.).

The size of the isotopic niche in each region was calculated in the package SIAR (Parnell and Jackson, 2011) in R version 3.0.2 (R Development Core Team, 2014; <http://www.r-project.org>) as standardized ellipses (SEAc) that incorporate the 40% densest data points within a dataset (Jackson et al., 2011). This approach has been developed to particularly account for small and variable sample sizes (Parnell et al., 2010). Regional differences in isotopic niche size between different regions were analyzed by comparing probability distributions from Bayesian standard ellipses with 95% credible intervals of isotopic niches. Effects of land-use intensity on isotopic niche size were analyzed by PERMANOVA (same model structure as previously described) on normalized $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values in a joint model.

RESULTS

Abundance

The abundance of three out of four detritivorous mesofauna species (*Eupelops occultus*, *Tectocephus velatus*, and *Isotoma viridis*) was significantly related to land-use intensity, but the strength and direction of this relationship depended on the study region (Table 2). Abundances of all three species increased with land-use intensity in the southernmost region and decreased with land-use intensity in the central region (Figures 1A–C). In the northernmost region, the abundance of the collembolan species *I. viridis* increased with land-use intensity (Figure 1C), while that of the oribatid mite *E. occultus* decreased with an increase of land-use intensity (Figure 1A). Regional differences in the abundance of the two centipede species *Geophilus flavus* and *Schendyla nemorensis* (Chilopoda, Geophilomorpha) were very similar (Figures 2A,B), with the significant main effects of the factor “Region” on this parameter (Table 2) largely reflecting very low densities in the northernmost region and highest densities in the central region. The millipede species *Cylindroiulus caeruleocinctus* (Diplopoda, Julida) also had highest densities in the central region, but did not show very low densities in the

northernmost region (Figure 2C). While no abundance effect of land-use intensity could be established for *G. flavus*, the abundance of *S. nemorensis* declined with land-use in all regions (Figure 3A). No significant abundance effect of region or land-use has been found for Parasitidae and the oribatid species *Tricoribates trimaculatus* (Table 2).

Isotope Values

Stable isotope analysis clearly separated the investigated taxa into primary consumers (oribatid mites, *I. viridis*, *C. caeruleocinctus*) with low $\Delta^{15}\text{N}$ ratios and secondary consumers (centipedes, Parasitidae) with high $\Delta^{15}\text{N}$ ratios (Figure 4). The fact that the $\Delta^{13}\text{C}$ ratio of Parasitidae was in the same low range as those of detritivorous microarthropods (oribatid mites, *I. viridis*) indicates that this taxon selectively feeds on small soil animals located close to the basis of the decomposer food chain. The high $\Delta^{13}\text{C}$ ratio of *C. caeruleocinctus*, in contrast, suggests the use of carbon from living plants. Though the intermediate $\Delta^{13}\text{C}$ ratios of the two centipede species seem to indicate a somewhat unspecific feeding behavior, these values might thus also reflect the predation on soil animals with different feeding preferences (Figure 4).

The fact that no impact of land-use intensity or region on the $\Delta^{15}\text{N}$ ratios was found proves that external factors do not fundamentally change the trophic level of the eight soil taxa investigated (Table 2). Significant PERMANOVA results for the $\Delta^{13}\text{C}$ ratios of two taxa indicate, however, that trophic shifts may occur. The $\Delta^{13}\text{C}$ ratio of the centipede *S. nemorensis* was negatively related to land-use (Figure 3B). Significant interactions (Table 2) additionally show that the $\Delta^{13}\text{C}$ ratio of this species was comparatively low in the central region and strongly declined with increasing land-use intensity in the northern- and southernmost regions (Figure 3B). Significant regional differences in the trophic behavior could be established for the oribatid mite *T. trimaculatus* (Table 2). The $\Delta^{13}\text{C}$ ratio of this species was higher in the southernmost region than in the other two regions (AEG 1.9 ± 0.6 vs. HEG 1.3 ± 0.3 and SEG 1.4 ± 0.7).

The overlap of isotope values for plant samples was high between the southernmost and central region, but generally lower with the northernmost region (Figure 5). The overlap in isotopic niches within species and between study regions was low for *I. viridis* (Figure 6C) and *E. occultus* (Figure 6B), but comparatively high for Parasitidae (Figure 6A), centipedes (Figures 6D,E), and millipedes (Figure 6F), respectively. Regional differences in the size of isotopic niches were confined to Parasitidae (Figure 7A: AEG vs. SEG: $P = 0.031$) and *E. occultus* (Figure 7B: HEG vs. SEG, $P = 0.042$). A significant effect of land-use intensity on the size of isotopic niches could only be established for the centipede *S. nemorensis* [$F_{(1, 41)} = 5.52$, $P = 0.008$].

DISCUSSION

This study aimed at developing a framework for the effect of regional conditions and land-use intensity on the soil fauna as a basis for predicting spatial variation in decomposition- and

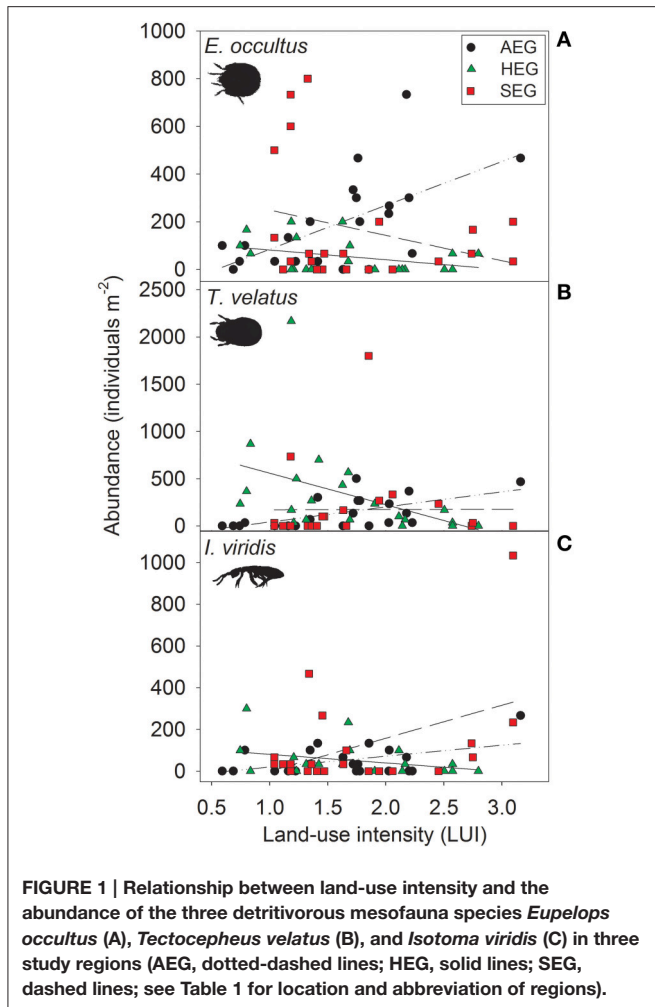
TABLE 2 | Results of permutational analyses of variance with the fixed factor “Region” and the continuous predictor “Land-use intensity” (mean LUI 2008–2010, Blüthgen et al., 2012) for (a) abundance, (b) resource utilization ($\Delta^{13}\text{C}$), and (c) trophic level ($\Delta^{15}\text{N}$).

	Mesostigmata						Oribatida					
	Parasitidae			<i>E. occultus</i>			<i>T. velatus</i>			<i>T. trimaculatus</i>		
	df	F	P	df	F	P	df	F	P	df	F	P
ABUNDANCE												
Land-use intensity	1	2.20	0.138	1	4.34	0.037	1	0.23	0.632	1	0.33	0.565
Region	2	0.18	0.912	2	44.35	<0.001	2	153.70	<0.001	2	1.97	0.374
LUI × Region	2	1.32	0.517	2	62.54	<0.001	2	114.77	<0.001	2	3.45	0.178
Residuals	57			57			57			57		
$\Delta^{13}\text{C}$												
Land-use intensity	1	0.01	0.931	1	0.22	0.657	1	4.38	0.065	1	3.14	0.091
Region	2	0.33	0.721	2	1.29	0.310	2	0.14	0.875	2	5.12	0.016
LUI × Region	2	0.10	0.391	2	0.38	0.700	2	2.37	0.178	2	3.37	0.056
Residuals	37			19			5			14		
$\Delta^{15}\text{N}$												
Land-use intensity	1	0.53	0.473	1	0.01	0.915	1	0.18	0.697	1	0.01	0.802
Region	2	0.01	0.921	2	0.01	0.956	2	1.07	0.410	2	0.01	0.915
LUI × Region	2	0.01	0.935	2	0.46	0.605	2	1.78	0.250	2	0.12	0.887
Residuals	37			19			5			14		
	Collembola			Chilopoda						Diplopoda		
	<i>I. viridis</i>			<i>G. flavus</i>			<i>S. nemorensis</i>			<i>C. caeruleocinctus</i>		
	df	F	P	df	F	P	df	F	P	df	F	P
ABUNDANCE												
Land-use intensity	1	6.28	0.012	1	0.55	0.458	1	17.36	<0.001	1	3.77	0.052
Region	2	23.65	<0.001	2	18.30	<0.001	2	15.04	<0.001	2	9.50	0.009
LUI × Region	2	29.94	<0.001	2	2.95	0.229	2	4.29	0.117	2	3.94	0.139
Residuals	56			144			144			144		
$\Delta^{13}\text{C}$												
Land-use intensity	1	0.01	0.824	1	0.01	0.824	1	9.71	0.004	1	3.04	0.084
Region	2	0.89	0.456	2	0.38	0.686	2	4.13	0.027	2	0.72	0.490
LUI × Region	2	0.1	0.434	2	0.32	0.725	2	3.61	0.038	2	0.36	0.693
Residuals	5			45			36			45		
$\Delta^{15}\text{N}$												
Land-use intensity	1	0.57	0.503	1	0.01	0.862	1	1.50	0.232	1	1.16	0.294
Region	2	0.32	0.754	2	0.46	0.631	2	0.48	0.622	2	1.03	0.364
LUI × Region	2	1.98	0.217	2	0.33	0.726	2	0.01	0.932	2	1.05	0.358
Residuals	5			45			36			45		

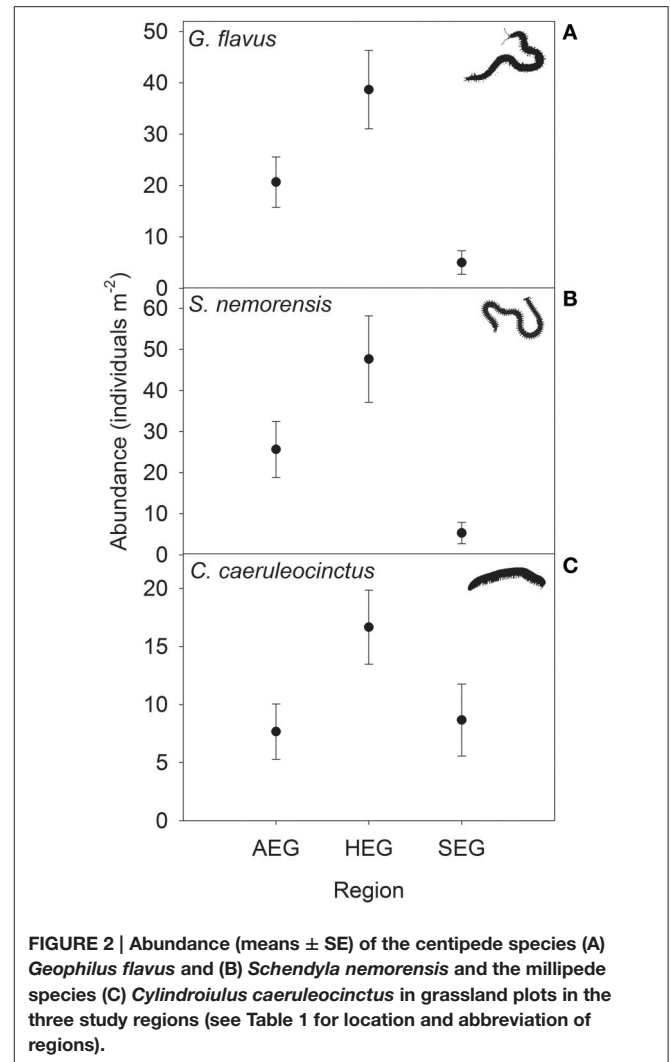
All isotope ratios are baseline-corrected for local plant resources. Significant model terms are bold.

predation-related ecosystem services (cf. Zhang et al., 2008). Against this background, our results allow us to assort the investigated taxa to three groups: (1) numerical response only (*T. velatus*, *G. flavus*, *C. caeruleocinctus*), (2) numerical and trophic response (*E. occultus*, *I. viridis*, *S. nemorensis*), and (3) trophic response only (Parasitidae, *T. trimaculatus*). Thus, alterations in the ecosystem services provided by soil animal communities are due to complex shifts in the abundance and/or functional role of all species. Notably, our grouping does not resemble any of the established taxonomic or soil ecological classification

systems (such as meso- vs. macroarthropods or detritivores vs. carnivores; Coleman et al., 2004). It thus provides an alternative framework for the evaluation of environmental effects on soil animals. Obviously, such a very coarse scheme needs to be more elaborated for becoming operational, for example by considering dominance structures of soil animal communities including abundance data for less common species. Fine-tuning is possible by additionally considering the direction of the numerical response as well as the type of the trophic response (change of the basal food source vs. trophic level shift vs. variations



in isotopic niches). The numerous significant effects of the factors “Region” and “Land-use intensity” on these parameters suggest that the region-specific reaction of the soil fauna to human intervention can at least partly be included in predictive approaches to ecosystem services via spatially explicit modeling (Ettema and Wardle, 2002). This, of course, would require to better understand the response of species that critically impact the provision of soil ecosystem services to regional conditions. Considering the regional differences in the isotope signatures of plants found in this study (cf. Kleinebecker et al., 2014), our approach of correcting soil animal values for this bias seems to be crucial in this context. However, the successful identification of large-scale relationships between environmental parameters and soil biota (Fierer et al., 2009; Decaens, 2010; Birkhofer et al., 2012) suggests that it is very well possible to establish region-specific response-patterns for informing predictive models. Though this is a very ambitious task, it might be an inevitable consequence of our findings that warn us against too broad generalizations of results gained from investigations carried out in single case study regions. Since our study was confined to eight common taxa, however, such far-reaching conclusions are preliminary and only intend to advance the conceptual framework mentioned above.



The impact of land-use on the abundance of the microarthropods assembled in groups 1 (numerical response only) and 2 (numerical and trophic response) was significantly different among regions. This confirms that the effects of grassland management on soil mesofauna is strongly context specific (Curry, 1994). The fact, however, that the direction of the response to land-use intensity was identical for all species of these two groups in two out of three regions suggests a quite predictable impact of human intervention on detritivorous microarthropods. This conclusion is further supported by the fact that land use did not induce a trophic response of any of the microarthropod taxa investigated, i.e., major changes in the functional role do not have to be considered. Regional differences in dietary niche size and overlap nevertheless indicate a substantial influence of geographic conditions on food exploitation by most microarthropods (cf. Crotty et al., 2014). Concerning the detritivorous mesofauna, however, a significant regional shift of the basic food source could only be established for the oribatid mite *T. trimaculatus* (group 3, trophic response only). This species often colonizes lichens covering

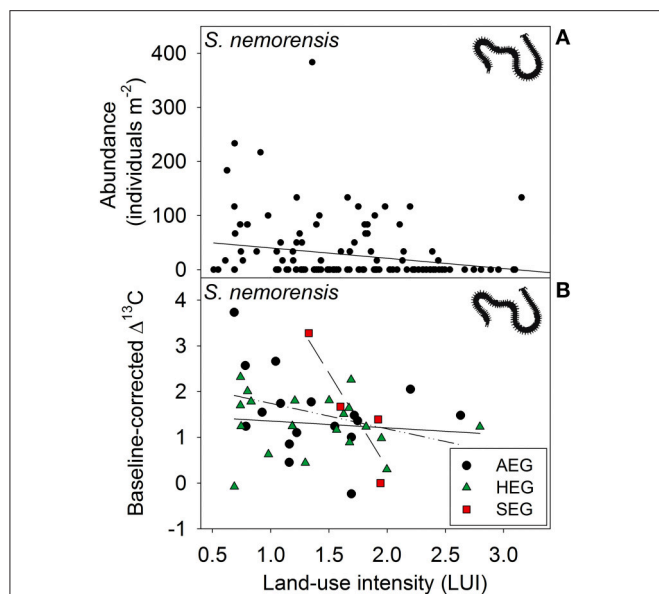


FIGURE 3 | Relationship between land-use intensity and the (A) abundance and between land-use intensity per region and the (B) $\Delta^{13}\text{C}$ isotope ratio (AEG, dotted and dashed line; HEG, solid line; and SEG, dashed line) in the centipede species *Schendyla nemorensis* (see Table 1 for location and abbreviation of regions).

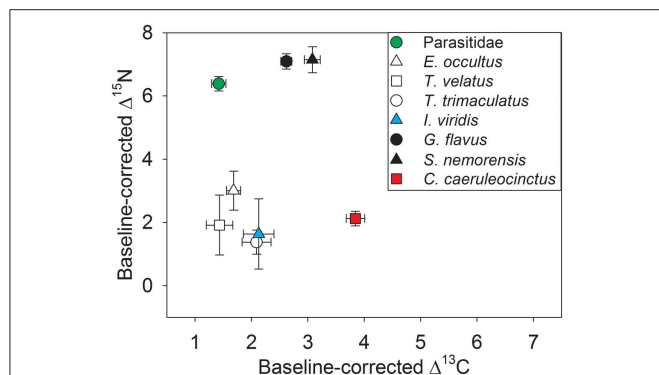


FIGURE 4 | Carbon and nitrogen stable isotope ratios (means \pm SE) for soil arthropod species with the stable isotope signature of plant samples at each site used as local baseline (Δ values). The closer an average Δ value (^{13}C or ^{15}N) is to zero on the carbon or nitrogen axis the less enriched the respective species is compared to isotopic values of plant samples from the same sampling site. For sample sizes see Table 1.

tree trunks (Graczyk and Seniczak, 2013) and is known to be ecologically quite opportunistic. Thus, one might speculate that the abundance of this species remained quite constant, because it compensates for regional difference in resource availability by a fundamental shift in the feeding behavior, i.e., a switch from plant litter- to algae- or lichen-based diets. Such a response has already been observed for other soil detritivores (Crotty et al., 2014). Yet, the fact that the $\delta^{15}\text{N}$ signatures of all taxa included in our study exceeded those of higher plants indicates that algae

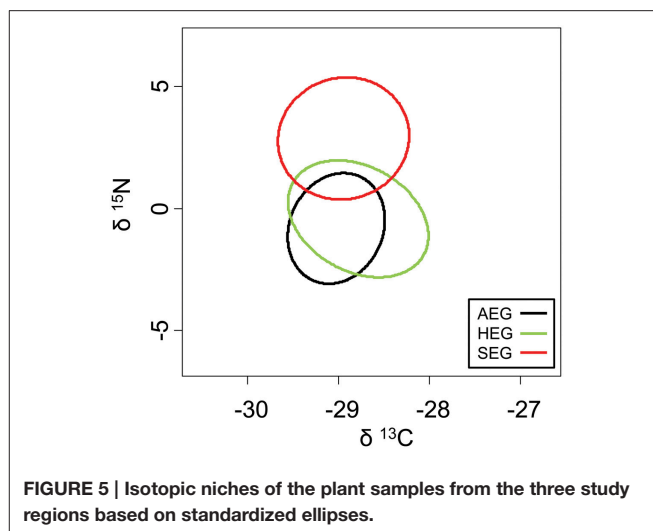


FIGURE 5 | Isotopic niches of the plant samples from the three study regions based on standardized ellipses.

or lichens are not among the major food sources of grassland arthropods (Schneider et al., 2004; Tiunov, 2007).

The millipede *C. caeruleocinctus* (group 1, numeric response only) was far less depleted in ^{13}C than detritivorous microarthropods. This could be explained by the utilization of root derived carbon (Klarner et al., 2013; Ferlian et al., 2015). Since this species can become a pest in sweet potato fields (Brunke et al., 2012), one might doubt the contention that millipedes have a solely beneficial role in grassland ecosystems. Though millipedes definitely contribute to litter fragmentation and structuring of soil in a wide range of ecosystems (i.e., are “ecosystem engineers” *sensu* Jones et al., 1997, see also Hättenschwiler et al., 2005; Hauser and Voigtländer, 2009), several species—similar to some Collembola (Endlweber et al., 2009)—may cause ecosystem disservice by consuming living plant material.

The two centipede species in our study are also listed in the numerical response groups 1 (numerical response only) and 2 (numerical and trophic response). Both species belong to the predaceous soil macrofauna, often occur in open habitats (Bonato et al., 2005) and hunt in the uppermost soil layers as well as on the soil surface (Wolters and Ekschmitt, 1997; Lewis, 2008). High densities in permanent grasslands of the southernmost and of the central study region indicate that centipedes may significantly affect ecosystem services by both preying on decomposer organisms and regulating soil-associated grassland pests (cf. Voigtländer and Düker, 2001). The intermediate $\Delta^{13}\text{C}$ ratios of centipedes found in our study support the conclusion that Geophilomorpha preferentially feed on prey that consume primary carbon sources derived from roots (Tiunov, 2007) such as certain insect larvae (e.g., Diptera, Coleman et al., 2004). Since rhizophages are among the most severe pests in Central European grasslands (Alford, 2011), our results point to the potential influence of grassland centipedes on the control of belowground herbivores. Though centipedes are appreciated by some gardeners, their contribution to this essential ecosystem

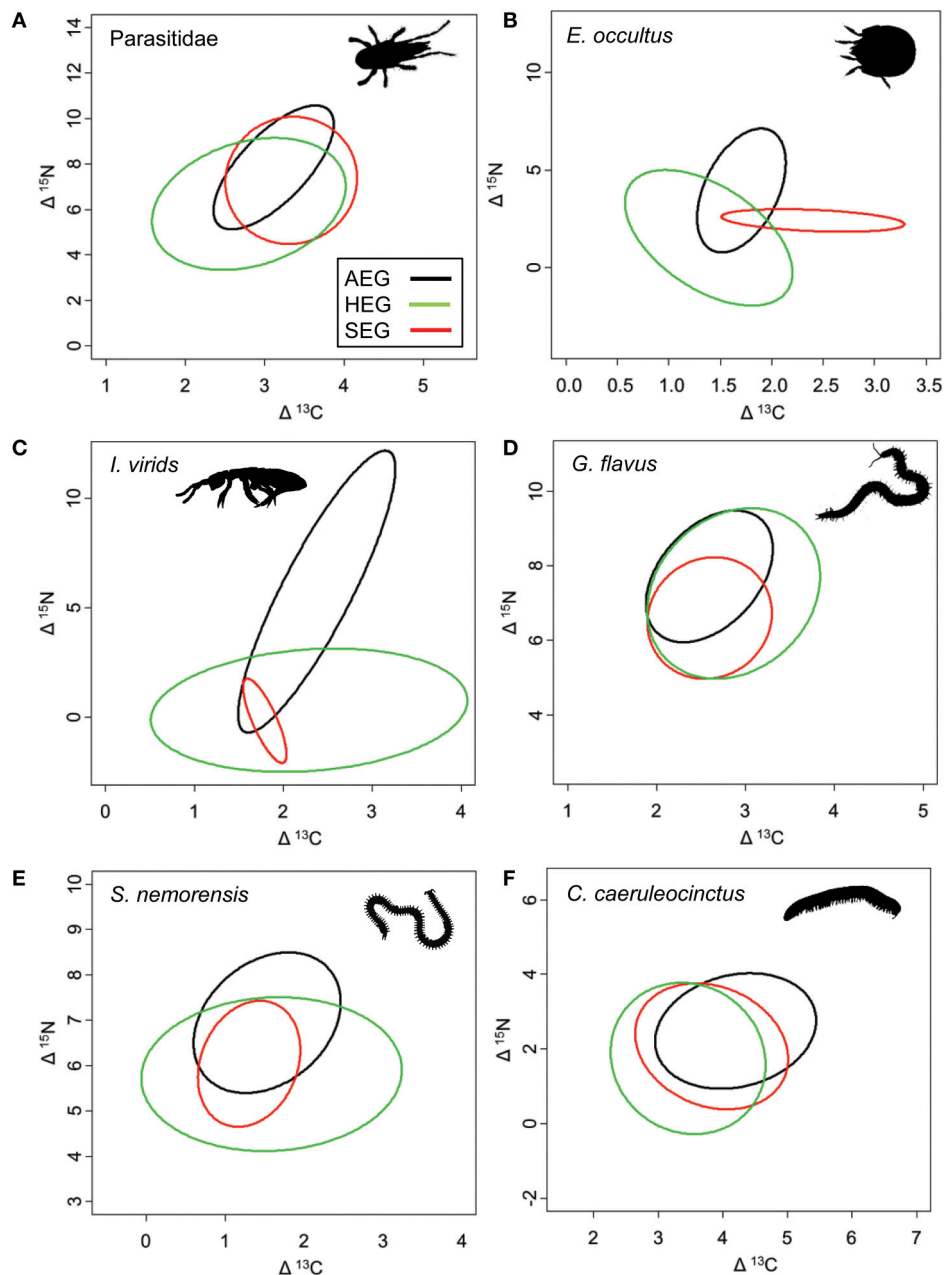
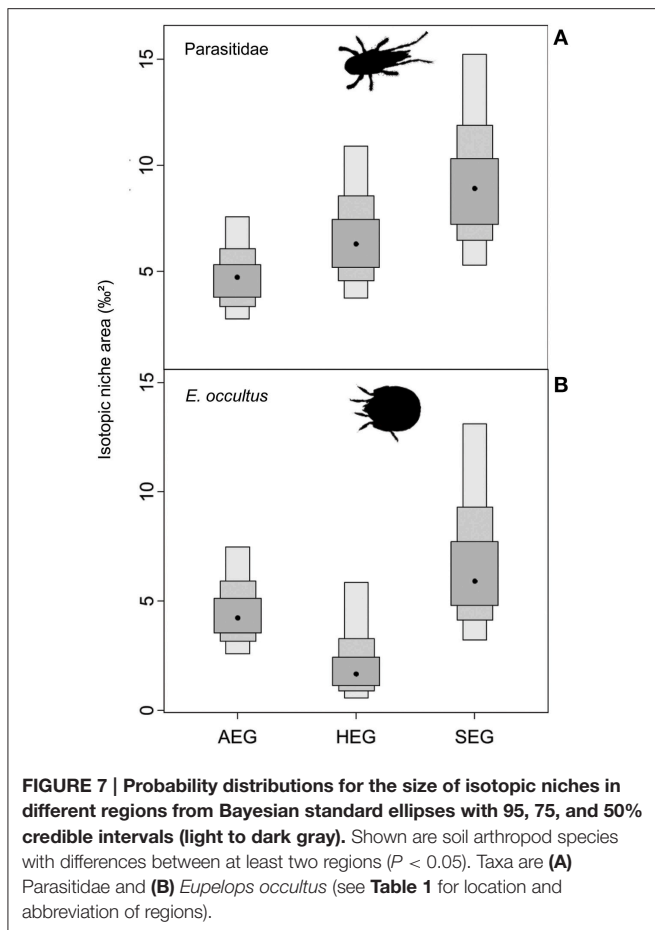


FIGURE 6 | Isotopic niches of the six soil arthropod species that were sufficiently common to calculate standardized ellipses for the feeding niches in all three study region (occurrence in a minimum of three sites per region; see Table 1 for location and abbreviation of sites). Note that standardized ellipses account for different sample sizes per region and are adjusted for small sample sizes (SEAc; Jackson et al., 2011). Taxa are (A) Parasitidae, (B) *Eupelops occultus*, (C) *Isotoma viridis*, (D) *Geophilus flavus*, (E) *Schendyla nemorensis*, and (F) *Cylindroiulus caeruleocinctus*.

service in permanent grasslands might have been underestimated in the past (see also Poser, 1990).

Low abundance of centipedes in the northernmost region most probably results from the adverse effects of both unfavorable soil conditions (Barlow, 1957; Wolters and Ekschmitt, 1997) and reduced availability of important food sources (in particular earthworms; Birkhofer et al., 2012). Though our findings indicate a very consistent effect of regional

conditions on centipede abundance, the trophic response of the two species to land-use intensity was very different. *G. flavus* which is fairly well adapted to anthropogenic habitats (Bonato et al., 2005) does not seem to respond to this factor at all. In contrast, the eurytopic species *S. nemorensis* (Berg and Hemerik, 2004) declined in abundance with land-use intensity and also showed a significant trophic response to land-use intensity. Decreasing $\Delta^{13}\text{C}$ values of *S. nemorensis* with increasing land-use



intensity suggest a shift of prey from rhizophages to saprophages (Pollierer et al., 2009). Considering the different response patterns of the micro- and macrosaprophages investigated in our study to land use, this could be explained by an increased consumption of taxa that were not adversely affected by high management intensity. Centipedes of grassland habitats are known to switch between prey groups even within individual years (Juen and Traugott, 2007). Management may thus alter ecosystem services (biological control of root-herbivores) and disservices (reduction of decomposition processes) provided by sensitive centipede species. Trophic effects of land-use might be even more severe than indicated by our isotope analyses, since the decline of $\Delta^{13}\text{C}$ values could have been partly masked by the fact that Geophilomorpha consume considerable amounts of earthworms (Lewis, 2008), which often have high $\delta^{13}\text{C}$ ratios (Pollierer et al., 2009).

Comparatively low $\Delta^{13}\text{C}$ values of Parasitidae (group 3: trophic response only) confirm that these predaceous microarthropods primarily consume species that feed on decomposing litter (Klarner et al., 2013). However, our finding that this taxon adjusts its trophic niche to regional conditions also suggests a rather flexible feeding behavior of this taxon (Curry, 1994). Trophic flexibility most probably contributed to the ability for maintaining similar abundance levels under all of the environmental conditions covered in our study. Due to the

coarse taxonomic resolution, the flexible response of Parasitidae can be explained either by a change in species composition or by a trophic response of individual species. However, in contrast to the classification suggested by Klarner et al. (2013) for forest ecosystems the trophic niche of Parasitidae inhabiting grassland soils significantly differed from that of the centipedes discussed above. This highlights the fact that very strong changes in ecosystem structure (such as the conversion of deciduous forests to permanent grasslands) may substantially alter the functional role of several taxa in belowground communities. Thus, findings on the feeding behavior and trophic position of edaphic animals pooled to coarse taxonomic groups cannot simply be extrapolated from one ecosystem to the other.

To conclude, the combined investigation of numerical and trophic response patterns gave us a differential insight into the impact of land-use and regional conditions on edaphic arthropods. Though stable isotope analyses have become quite popular in soil ecology over the last decades, this approach has rarely been used to complement the numerous comparative studies on the abundance of the belowground fauna. Considering that this new classification system is derived from the several consistent and significant effects of management and geographic context on the abundance and feeding behavior of soil arthropods, this seems to be a serious shortcoming. We are confident that this integrative approach provides a promising framework for designing spatially explicit models that quantify the impact of human interventions on the delivery of ecosystem services by the soil fauna.

AUTHOR CONTRIBUTIONS

KB designed the study, participated in the sampling, supervised identification, analyzed the data, developed the paper, and wrote the manuscript. CD, KJ, and QS participated in the sampling, identified sample material, analyzed the data, and contributed to the manuscript writing. AZ supervised identification and wrote the manuscript. VW designed the study, developed the paper and wrote the manuscript.

ACKNOWLEDGMENTS

We appreciate comments of two referees to an earlier version of this manuscript. We thank the managers of the three Exploratories, Kirsten Reichel-Jung, Swen Renner, Katrin Hartwich, Sonja Gockel, Kerstin Wiesner, and Martin Gorke for their work in maintaining the plot and project infrastructure; Christiane Fischer and Simone Pfeiffer for giving support through the central office, Michael Owonibi for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Jens Nieschulze, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser, and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. The work has been (partly) funded by the DFG Priority Program 1374 “Infrastructure-Biodiversity-Exploratories” (Refno. WO670/7-2). Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

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Conflict of Interest Statement: 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.

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Biomass and Diversity of Soil Mite Functional Groups Respond to Extensification of Land Management, Potentially Affecting Soil Ecosystem Services

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OPEN ACCESS

Edited by:

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Greece

Reviewed by:

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University of Warwick, UK
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Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Environmental Science

Received: 30 October 2015

Accepted: 25 February 2016

Published: 15 March 2016

Citation:

de Groot GA, Jagers op Akkerhuis GAJM, Dimmers WJ, Charrier X and Faber JH (2016) Biomass and Diversity of Soil Mite Functional Groups Respond to Extensification of Land Management, Potentially Affecting Soil Ecosystem Services. *Front. Environ. Sci.* 4:15. doi: 10.3389/fenvs.2016.00015

Soil mites (Acari) are ubiquitous in soil ecosystems and show a vast taxonomic diversity with a wide range of life history characteristics and feeding strategies. Various taxa contribute directly or indirectly to soil processes, including nutrient cycling, soil formation and pest control. Mites thus support important ecosystem services of soils. Yet, their community composition, and therewith service provisioning, may differ between for instance intensively managed agricultural soils and extensively managed grassland soils. We therefore hypothesized that successional changes in the abundance and diversity of soil mite functional groups (feeding types) will occur following a conversion of arable land to grassland, affecting their contribution to ecosystem services. To test this, we studied the succession of mite communities on two Long Term Observatories (LTOs) in Lusignan (France) and Veluwe (the Netherlands). At Lusignan, sampling involved four combinations of recent and historic land use types. At the Veluwe, samples were taken in a secondary succession chronosequence in grasslands, representing a time frame up to 29 years after the conversion from arable land to grassland. Biodiversity and biomass were higher in grassland than in arable land, especially for the total mite community, the predators and the main taxa aiding in decomposition. After conversion of grassland to arable land, or vice versa, both taxon richness and biomass rapidly developed towards the prevailing conditions. Our results indicated that the taxon richness and biomass of the total mite community in grassland still continued to increase up to 29 years after the conversion from arable land to grassland. Total taxon richness increased with time since conversion, which was mainly due to the immigration of decomposers and predators. The biomass of different feeding guilds increased at variable speeds. The observed changes imply an increase in nutrient cycling and in the suppression of some potential pests. We discuss the relevance of these ecosystem services in extensively managed grasslands and agricultural systems. Furthermore, our results suggest that in agricultural rotational schemes that include one or more years of grassland, mite communities and associated ecosystem services may be partially, but not completely, restored to the conditions of long term grassland.

Keywords: Acari, functional diversity, ecosystem services, secondary succession, pest control

INTRODUCTION

The average soil contains a huge taxonomic diversity of bacteria, protozoa, fungi, nematodes, enchytraeids, insect larvae, and earthworms. With their activity, all these organisms contribute to processes in the soil, either directly, for example through the digestion of plant roots or fungal hyphae, or indirectly, through the dispersal of bacteria and/or fungal spores. Viewing the process of ecosystem engineering as any physical transformation in the environment that modifies the resources for other organisms (Jones et al., 1994), soil invertebrates mediate soil functioning through a wide range of engineering processes. Examples of such processes are the redistribution of organic matter, bioturbation, the comminution, and incorporation of litter into soil, contributions to structural porosity and the formation of soil aggregates through burrowing, casting and nesting activities and the feeding on microbial communities (Freckman et al., 1997; Lavelle et al., 2006; Barrios, 2007; Brussaard et al., 2007).

Through these activities, soil invertebrates contribute in a significant way to the delivery of ecosystem services by soils (Wall et al., 2010; De Vries et al., 2013). By influencing the interactions that develop among soil physical, chemical and biological processes, they particularly affect what in the Millennium Ecosystem Assessment (Millennium Ecosystem Assessment Panel, 2005) are viewed as “regulating” or “supporting” services. Important examples of such services (Haines-Young and Potschin, 2013) are the control of pest and diseases, the decomposition of organic matter and the resulting nutrient cycling and soil formation.

A large body of studies has led to the general realization that highly intensive agricultural practices may strongly impact belowground biodiversity (e.g., Tsiafouli et al., 2015) and may have detrimental effects on intermediate ecosystem services, such as pest control, decomposition and nutrient cycling (Foley et al., 2005; Millennium Ecosystem Assessment Panel, 2005; Kremen and Miles, 2012). Via the agri-environmental schemes under the EU's Common Agricultural Policy (CAP) farmers can receive support and funding to apply a less intensive management, e.g., by transforming part of their land into extensively managed grasslands. Yet, while long-term grasslands are known to show relatively high biodiversity and ecosystem services like nutrient cycling, there is a general lack of long-term studies showing whether and when service provision is enhanced following a transition to extensively managed grassland (Horrocks et al., 2014). A clear time lag may exist between the management change and the arrival of stable microbial communities (Buckley and Schmidt, 2001) and increases in nutrient cycling efficiency (e.g., Horrocks et al., 2014, 2016). Effects on soil metazoa may strongly differ per taxonomic group. Within 3 years after grassland restoration, Postma-Blaauw et al. (2012) observed the recovery of a diverse nematode community but an only partial restoration of the species richness of predatory mites and negative effects on enchytraeid worms. More extensive studies are required to study effects in these groups on abundance and taxon diversity over longer periods of time.

This study focuses on soil mites, which are ubiquitous in soil ecosystems and show a vast taxonomic diversity with a wide range of life history characteristics and feeding habits. As a result, they are able to inhabit a broad range of habitats. Yet, they are known to show a negative association with the physical and chemical disturbances and low levels of organic matter that normally occur in agricultural soils (Petersen and Luxton, 1982; El Titi, 1984; Siepel and Van de Bund, 1988). After land management extensification, release from disturbance and a build-up in the amount, complexity and diversity of organic matter are expected to result in an increase of soil mite diversity with successional time (Bardgett and Shine, 1999; Maraun and Scheu, 2000). It has been shown that microarthropods are generally more abundant in soils of extensively managed systems, such as grasslands, than in intensively managed arable soils (Giller et al., 1997; Minor and Cianciolo, 2007; Postma-Blaauw et al., 2010). Likewise, taxon diversity has been shown to be higher in grasslands than in intensive arable lands (Menta et al., 2011), resulting in an overall higher soil biological quality. Here, we therefore hypothesized that the transition from an agricultural system to an extensively managed grassland will result in clear successional changes in the abundance and taxonomic composition of soil mite communities, and therewith in their contribution to ecosystem services.

The most studied service to which soil mites contribute concerns the decomposition of organic matter in the soil from roots and from other living or dead organic sources in the soil (Curry, 1969; Swift et al., 1979; Hågvar and Kjondal, 1981; Neutel, 2001). This underlies the process of soil formation, as well as the flow of energy and nutrients to higher trophic levels (Koehler, 1999). Different feeding guilds of mites (Siepel and De Ruyster-Dijkman, 1993) contribute to these processes in different ways. An important contribution to the decomposition of organic matter is made by the fungivore and herbivore grazers. These guilds are capable of digesting cell wall material, and liberate nutrients from the recalcitrant cell wall material (Siepel and Maaskamp, 1994). A wider variety of mites contributes to soil formation, through burrowing, through the fragmentation of organic material, through the production of feces (e.g., Van Vliet and Hendrix, 2007; Culliney, 2013). The fragmentation of organic material and the production of feces are typically accounted for by the fungivore grazers and herbofungivore grazers (Siepel and Maaskamp, 1994). In addition, fungal grazers and browsers may, through their feeding activity play a role in increasing soil aggregate stability in former arable land (Seastedt, 1984; Duchicela et al., 2013). Based on these contributions, changes in the diversity and (relative) abundances of mite feeding guilds after a transition from arable land to grassland can be expected to impact soil formation and nutrient cycling.

A number of previous studies indicate that changes in land use can also affect the abundance and species composition of predatory mites. Postma-Blaauw et al. (2012) showed that in arable land the numbers, and the taxonomic diversity of mesostigmatid mites (which includes the predatory taxa) were low, while in grassland more taxa were found, and in higher numbers. Mite communities of long-term and new arable land were very much alike, whilst markedly different from

those of long-term and new grasslands. The composition of mesostigmatid mites has also been found to clearly change with time since after a major land use change, for instance between young, mid-aged, and old fallows (Wissuwa et al., 2012) and in reclaimed power plant waste dumps (Madej and Stodółka, 2008). When combined, such results suggest a strong and immediate reducing effect of intensive soil treatment (e.g., plowing) and a slow succession from an arable related species composition to a long-term grass related species composition. In contrast to their low biomass, predatory mites can have a disproportionately high effect on matter flows in ecosystems (Moore and De Ruiter, 2000). Various taxa have been linked to pest control, either as natural predators (Afifi and Van der Geest, 1984; Koehler, 1999; Menzler-Hokkanen, 2006) or as inoculation biocontrol agents for e.g., bulb mites, thrips, and endoparasitic nematodes (Bennison et al., 2002; Gerson et al., 2003).

Despite of these general insights, only limited knowledge is available on the variation in the presence of different functional groups of mites across soils that are subjected to different management types. Even less is known about how the presence of these groups change following land use changes, and how this affects the provision of ecosystem services. Additional insights in these respects will improve our ability to optimize agricultural practice by making use of the natural diversity available in soils. In this study, we made use of the experimental set-ups available from two Long Term Observatories (LTOs), to observe successional patterns in soil mite communities (a) after a recent conversion of arable land to grassland, while taking historic land use into account, and (b) in a chronosequence of grasslands that differed in the period since conversion from arable practice. We assessed the (rate of) change in both abundance and taxonomic richness in the total community, as well as in various functional groups (feeding guilds), in order to derive hypotheses on the potential effect of a change in land use from arable land to grassland on soil functioning and associated ecosystem services.

MATERIALS AND METHODS

Site Description

The experimental results originate from two long-term observatory sites (LTO's) which have been part of the EU programme Ecofinders: Veluwe (the Netherlands) and Lusignan (France).

The SOERE "Agro-ecosystems, biogeochemical cycles and biodiversity" (ACBB) Lusignan experiment (INRA Poitou-Charentes, 2015) has been set up as a block design, consisting of four blocks, each subdivided in multiple fields. In three periods between 1996–2000, 2001–2004, and 2005–2011 the fields were cultivated in the following way: 1. permanent arable fields (AAA), 2. arable lands converted to grassland 7 years before sampling (AAG), 3. grasslands that were converted to arable land for 4 years, after which they were converted again to grassland 7 years before sampling (GAG), and 4. Grasslands which were converted to arable land and remained arable land during the 11 years before sampling (GAA). A schematic overview of the land use history per block is presented in Figure S1.

The Veluwe LTO site included nine fields at different locations. All the fields were situated on sandy soils with similar characteristics, and had been in use as arable land in the past. Arable fields were converted to grassland at different points in time (varying from 6 to 29 years before the moment of sampling; see Table 1) and represent a chronosequence of fields with different successional ages.

Sampling and Identification

Each of the four land use history types at the Lusignan LTO was represented by two replicate fields. Four soil cores were sampled in each field (summed surface of 105,58 cm², depth from 0 to –10 cm) and the extracted mites were pooled into one sample per field. In this way a total of eight samples was obtained representing four land use combinations times two replicates.

At the Veluwe LTO, three plots were randomly located in each of the nine fields of the chronosequence. In each plot, a soil core was sampled (surface 26.42 cm², depth from 0 to –10 cm) which was separately extracted for mites. In total, 27 (nine times three) samples were collected.

Soil cores were obtained with the help of a split-corer, filled from top to bottom with 2.5 cm high plastic rings (according to ISO standard 23611-2-2006; ISO, 2006). The diameter and depth of a sample was 5.8 cm and 10 cm (four rings), respectively. The volume of a sample was 264.2cc. The samples were transported to the lab in the PVC rings in PVC tubes closed with lids on the bottom and top, while kept in a cool-box. The mesofauna was extracted by means of Tullgren extraction for 7 days (ISO, 2006). A two-step extraction was used, with a 3-days initial temperature of 28°C and a subsequent 4-days period of 45°C, using a heat generating carbon wire light bulb (60W). The obtained micro-arthropod communities of the three samples per plot were pooled, and stored in 97% ethanol.

A standardized gel-based subsampling method (Jagers op Akkerhuis et al., 2008) was used to identify a random subsample of 150 individual mites per pooled sample. Taxon identification was based on (Weigmann, 2006) for Cryptostigmata (Oribatida), (Karg, 1989), Karg (1993) for Mesostigmata, Krantz and Walter

TABLE 1 | Site characteristics for the nine fields of the Veluwe LTO chronosequence, with respect to location (UTM coordinates) and successional age: the year of conversion to grassland, and between brackets the number of years between the conversion and sampling for this study.

Site name	Latitude	Longitude	Year of conversion
Dennenkamp	52° 01' 44.5894"	005° 48' 03.5091"	1982 (29)
Mosselsche Veld	52° 04' 24.2422"	005° 44' 13.9967"	1985 (26)
Wolfheze	51° 59' 47.7078"	005° 47' 37.1111"	1988 (23)
Nieuw Reemst	52° 02' 34.1978"	005° 46' 29.5062"	1990 (21)
Mossel	52° 03' 39.1373"	005° 45' 06.8209"	1995 (16)
Loenen	52° 04' 32.3026"	005° 57' 41.2815"	1999 (12)
Telefoonweg	52° 00' 08.7991"	005° 45' 11.0326"	2002 (9)
Oud Reemst	52° 02' 26.0863"	005° 48' 35.9526"	2005 (6)
Reyerscamp	52° 01' 00.1854"	005° 46' 21.3066"	2005 (6)

(2009) for Prostigmata and Hughes (1976) for Astigmata. Identification up to species level was attempted. Within some taxonomic groups, however, juveniles lack sufficient (known) morphological discriminatory characters. Such juvenile individuals could only be identified to genus or family level.

Biomass Calculations

Body length, width and height were measured for multiple specimens per taxon, available from our reference collection. Average sizes were calculated per taxon. For eight taxa we had no reference specimens and body sizes were obtained from the literature: *Ceratozetes minutissimus*, *Lucoppia burrowsi*, *Ameroseius corbiculus*, *Amerobelba decedens*, *Oppiella falcata*, *Protoribates capucinus* (Gil-Martín and Subías, 1998; Bruin et al., 1999; Murvanidze and Weigmann, 2003; Simsek and Baran, 2012; Jamshidian et al., 2015). Based on the body measurement data, the volume of “an average individual” was calculated for each taxon, assuming an ellipsoid shape for mites. A density of 1.1 g/cm³ was assumed for all taxa (pers. comm. Prof. Dr. H. Siepel). Based on the calculated densities per square meter, the total biomass was calculated per taxon per area (g/ha).

Statistical Analysis

Biomass and species richness were calculated for the total mite community per plot, as well as per feeding guild. Based on definitions by Siepel and De Ruyter-Dijkman (1993), we distinguished six main feeding guilds: bacteriovores, fungivores, herbofungivores, herbivores, predators, and omnivores. As physiological studies by Siepel and De Ruyter-Dijkman (1993) indicated marked differences between grazers and browsers, the fungivore and herbofungivore strategies were split into two subgroups. Fungivore and herbofungivore grazers can digest fungal cell walls, a property that is relevant for the decomposition of organic material (Siepel and Maaskamp, 1994). Therefore, an additional group was created in which the fungivore grazers and herbofungivore grazers were jointly named “main decomposers,” for which group separate values were calculated for the biomass and taxon richness (as a measure of diversity). Classification of mite taxa into the abovementioned groups was based on a database available at Alterra, established based on taxonomic information in combination with the broad range of ecological literature about feeding habits (e.g., Bhattacharyya, 1962; Hartenstein, 1962; Karg, 1962; Ito, 1971; Luxton, 1972).

For the Lusignan LTO, we tested for differences in both biomass and taxon richness per feeding guild using linear mixed models in SPSS version 22 (IBM corp.), with land use treatment as categorical fixed variable and block as random variable. With respect to taxon richness, we only analyzed differences for the six main feeding guilds, since a further differentiation resulted in too low numbers of taxa per group to produce valid statistics. In case of significant overall differences, LCD post-hoc tests were used to test for pairwise differences between treatments. Because we were mainly interested in the development of the mite community following a transition from arable land to grassland, and to reduce the number of pairwise comparisons, we focused on the differences between the fields that were converted to grassland but had different historic land use up to 11 years before our

sampling event (AAG and GAG; see Section Site Description for exact code descriptions), and the difference between each of those and the permanent arable fields (AAG–AAA and GAG–AAA).

For the Veluwe LTO experiment, effects of time since the transition to grassland on biomass and taxon richness were also tested using linear mixed models in SPSS, using time since transition as a continuous fixed variable and site as random variable. For this experiment, biomass data per feeding guild were log transformed prior to all analyses to comply with assumptions for normality. We tested separate models for the total community and each of the feeding guilds (again restricting to the six main guilds with respect to taxon richness). In some cases where no significant linear relation was found, mite biomass or taxon richness seemed to converge to a certain maximum value. Therefore, in such cases we also tested an inverse regression model ($y = b_0 + b_1/x$) and an S-curve regression model ($\ln(y) = b_0 + b_1/x$) via the curve estimation option in SPSS.

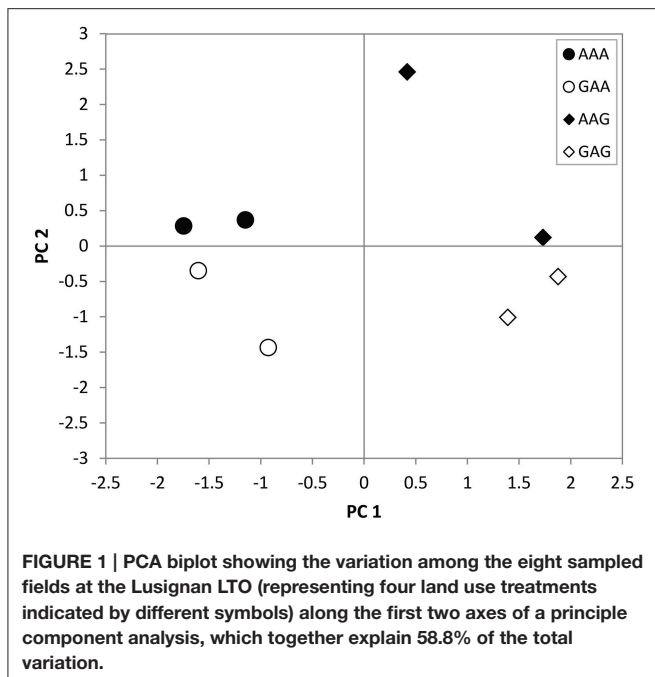
Differences in taxonomic composition among the nine fields of the Veluwe experiment, as well as among the fields with different land use treatments at Lusignan, were analyzed with the help of principle component analysis (PCA) in PCOrd (v.6.0; McCune and Mefford, 1999), based on presence/absence scores per taxon per site or field. To improve clarity in the PCA biplot for the Veluwe experiment, the fields were grouped in three classes of time since transition to grassland: recent, middle, and old. The following locations were associated with “recent”: Oud Reemst (2005), Reyerscamp (2005), Telefoonweg (2002) and Loenen (1999). The following locations were associated with “middle”: Wolfhezerheide (1988), Mossel (1995), and Nieuw Reemst (1990). The following locations were associated with “old”: Mosselsche Veld (1985) and Dennenkamp (1982). For the Lusignan experiment, fields were grouped according to historical land use treatment.

RESULTS

Lusignan Experiment

Based on the multivariate analysis, the mite community responded in different ways to the four land use history types at the Lusignan LTO (Figure 1). Firstly, the fields grouped along the first axis according to the most recent land use type. That is, the composition of the four fields that were converted to grassland for the last 7 years was relatively similar (AAG and GAG), while their composition was different from that of the fields that were arable land for the last 11 or 16 years (AAA and GAA). Secondly, fields were split along the second axis according to their historic land use type (GAA vs. AAA and GAG vs. AAG). Thus, a past difference in land use type still resulted in a difference in species composition after 11 years of equal land use.

The above trends with cropping system and time after conversion were largely reflected in the data of taxon richness and biomass. The largest differences in taxon richness and biomass of the total mite community were observed between the fields that shared the historic land use as arable field, but were recently converted to either arable land or grassland (AAA–AAG; Figure 2, Table 2). When considering the various feeding guilds, the predators, the fungivores and the herbivore



grazers showed a higher biomass in the fields that recently were converted into grasslands. For the predators, these differences were mostly explained by differences in the abundance of the general predators (Figure 3A). Amongst the fungivores, the fungal browsers showed a clear increase in biomass following conversion to grassland, while this increase was not significant for the fungal grazers (Figure 3B, Table 2). The group of main decomposers seemed to show a higher biomass in the recent grasslands (AAG and GAG) than in the recent arable lands (AAA and GAA) but this difference was insignificant due to large variation among the recent grassland plots (Figure S2, Table 2). Although in the multivariate analysis the recent grasslands (GAG and AAG) were separated from the other land use history types based on recent land use (indicating a difference in taxonomic composition), the biomass of the total community or of any specific feeding guild did not significantly differ between these land use history types (Table 2). Significant differences in taxon richness among any of the land use history types were found only for predators: arable fields recently converted into grassland showed a higher richness than permanent arable fields (Table 2).

Veluwe Chronosequence

The results of the multivariate analysis are shown in Figure 4. The variation among fields along the first axis is roughly in line with their age rank in the chronosequence. Recently converted fields clearly separated from the “old” grasslands. The middle plots showed a broad variation in species composition. The species composition of Mossel ranked between the old and new samples. However, the species compositions of Nieuw-Reemst and Wolfheze did not comply with the general pattern of temporal succession. Variation along the second axis mainly concerned differences in taxonomic composition between the

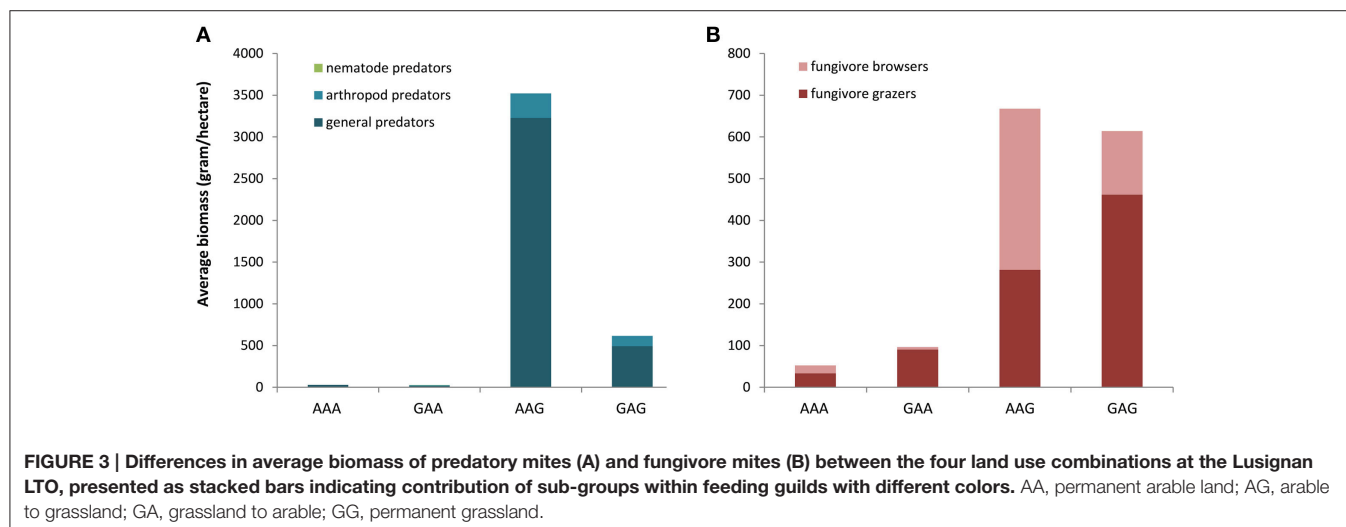
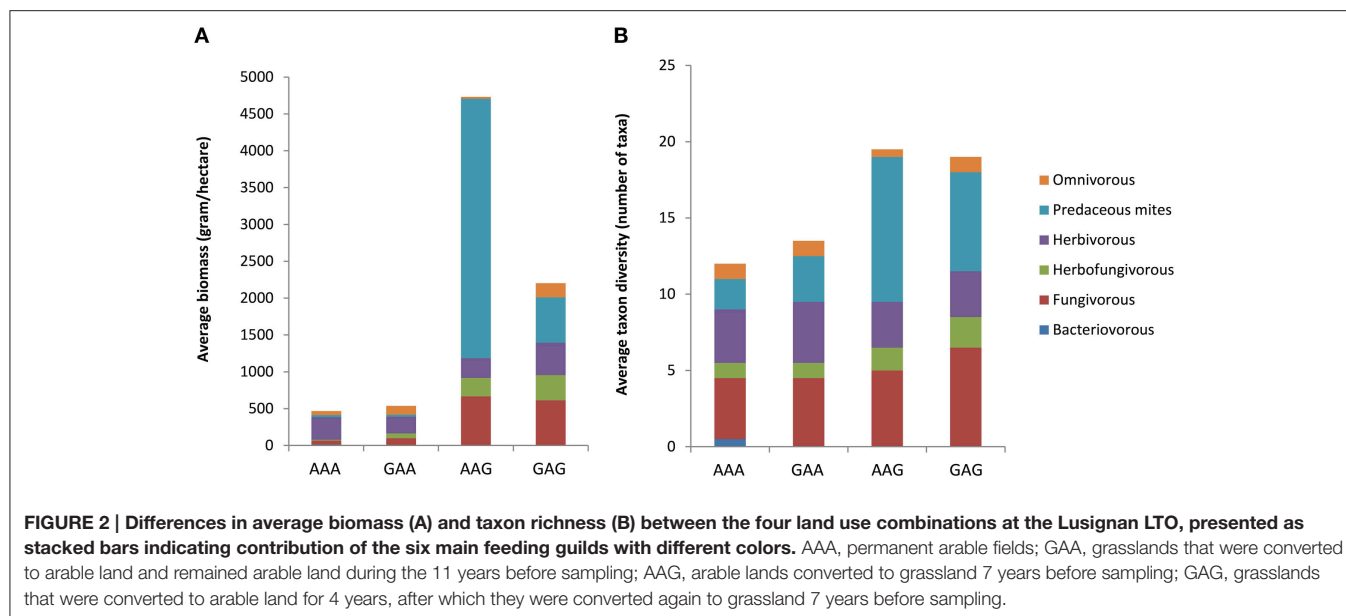
four recently converted fields. The “old” grasslands showed little variation along this axis.

Both the average biomass and taxon richness of the total mite community increased over time after conversion to grassland (Table 3, Figures 5A,B). When looking at individual functional groups, no linear increase was observed for the biomass of the predatory mites, but a significant inverse relation existed ($df = 1$; $F = 10.291$; $P = 0.004$), indicating a rapid increase in the first 5–10 years after the transition to grassland and slower development afterwards (Figure 5C). This relation was mainly accounted for by the general predators (significant inverse relation; $df = 1$; $F = 12.125$; $P = 0.002$), representing 83–98% of the total biomass of predatory mites (see Figure S3 for separate graphs for general, arthropod and nematode predators). A significant linear increase over time was found for the summed number of predatory taxa (Figure 5D). The biomass of all herbivores increased with time after conversion (Table 3), but this effect was almost entirely explained by a high biomass of one herbivore grazer (the oribatid mite species *Platynothrus peltifer*) in the oldest plot (Figure 5E). The herbivore browsers did not show a significant trend. The taxon richness of the herbivore community was relatively low in fields converted 6 years ago (Figure 5F), but remained stable from 10 years after the conversion onwards (significant inverse relation; $df = 1$; $F = 21.108$; $P = 0.003$). The biomass of all fungivores was only near significant ($P = 0.069$; Table 3, Figure 5G). The reason for this was that this group combines the fungivore grazers, which significantly increased over time (Table 3), and the fungivore browsers, which showed highly variable results (see Figure S4). The taxon richness of the fungivore community did not significantly change over time (Figure 5H). The average biomass and number of taxa of the main decomposers was positively related with time since the transition to grassland (Figures 5I,J).

DISCUSSION

Succession in Soil Mite Communities Following a Transition From Arable Land to Grassland

Both the diversity and the composition of microarthropod species assemblages in soils are known to differ substantially with land use type, and will respond to changes in land management (Minor and Cianciolo, 2007). In a general study on microarthropods, Menta et al. (2011) showed taxonomic assemblages to be clearly different between grasslands and agricultural lands, a pattern that was largely driven by differences in the mites and springtails. A similar distinction was observed for soil mites by Dirilgen et al. (2015) across a north-south European transect. Moreover, microarthropods are less abundant in intensively managed arable soils than in soils of extensively managed systems, such as grasslands (Giller et al., 1997; Postma-Blaauw et al., 2010; Menta et al., 2011). Thus, the transition from an agricultural system to an extensively managed grassland can be expected to result in clear changes in the mite community of the soil. Indeed, the results from both our field experiments highlighted that changes in land use lead to marked changes



in taxonomic composition and increases in total biomass and number of taxa.

The multivariate analyses for the Lusignan dataset indicated that the composition of the mite community in recently converted fields was largely determined by the most recent land use type. This is perhaps not so surprising when considering conversion from grassland to arable soil, since a sudden application of tillage and agrochemicals will trigger important changes in the soil (Ingham, 1985; Cortet et al., 2002; Van Capelle et al., 2012). However, our results also show that grasslands develop a recognizable signature within 7 years after a transition from arable land use.

The difference in composition that still existed between fields that shared the same land use for the last 11 years but differed in land use before that time (Figure 1) indicates that land use has long-term consequences for the successional patterns of

the local mite community. This does also fit with the observed differences in species composition along the chronosequence of early to late successional grasslands at the Veluwe LTO (Figure 3), where biomass and taxon richness continued to increase with the age of the grassland up to 29 years after the transition from arable land. This steady increase in richness was in line with a previous study by Kardol et al. (2009) for the same chronosequence. These results seem to be in contrast with those from the Lusignan experiment, where the biomass and number of taxa were largely determined by the recent shift to grassland, suggesting that they change markedly within the first years after the transition while no significant differences were observed between recent grasslands with a different historic land use type. This apparent rapid stabilization of the number of taxa may be related to higher possibilities for colonization, since here plots with different treatments were located closely

TABLE 2 | Results of linear mixed models for the Lusignan LTO experiment, testing for differences in biomass and taxon richness per soil mite functional group between fields ($N = 8$) representing four different combinations of current and historical land use management.

Variable	Group	df	F	P	AAA-GAG	AAA-AAG	GAG-AAG
Biomass	All mites	3	32.507	0.015		*	
	Bacterivores	3	1.000	0.535			
	All fungivores	3	9.079	0.029	*	*	
	Fungivore grazers	3	0.945	0.492			
	Fungivore browsers	3	18.382	0.001	*	*	
	All herbofungivores	3	9.513	0.066			
	Herbofungivore grazers	3	2.831	0.156			
	Opp. herbofungivores	3	2.527	0.196			
	All herbivores	3	0.380	0.774			
	Herbivore grazers	3	14.352	0.013	**	*	
	Herbivore browsers	3	1.191	0.419			
	All predators	3	18.127	0.009	*	**	
	General predators	3	11.824	0.019	*	*	
	Arthropod predators	3	93.837	<0.001	**	**	
	Nematode predators	3	1.000	0.535			
	Omnivores	3	1.415	0.362			
	Main decomposers	3	15.887	0.060			
Taxon richness (per sample)	Total	3	115.356	0.009		**	
	Bacterivores	3	1.000	0.535			
	Fungivores	3	3.467	0.202			
	Herbofungivores	3	3.667	0.222			
	Herbivores	3	3.667	0.121			
	Predators	3	18.424	0.009		*	
	Omnivores	3	1.000	0.455			
	Main decomposers	3	3.347	0.227			

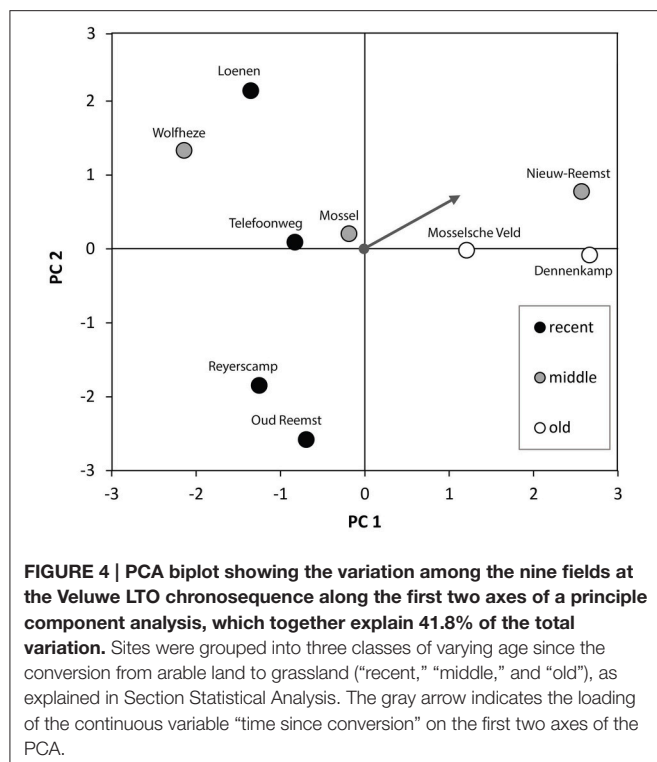
We present the result of the overall test between all four combination (df, F-value, and significance; bold text indicates significant difference at $P < 0.05$), as well as results of pairwise contrasts between permanent arable land (AAA), arable land converted to grassland (AAG) and permanent grassland with intermediate arable land rotation (GAG). The significance of the pairwise differences (as tested via LSD post-hoc tests) is indicated via asterisks, as follows: * $P < 0.05$; ** $P < 0.01$.

together in blocks, which may speed up the arrival of even slow dispersing taxa. Dispersal from adjacent habitats may thus be a dominant factor determining the development of the number of mite taxa following the establishment of a grassland. Relatively limited dispersal distances may also explain the marked variation in composition between the four young successional grasslands at Veluwe LTO, which is in line with the optimum in beta-diversity during the first years after the land use transition reported by Kardol et al. (2009). It can be hypothesised that the taxonomic composition of a grassland will be strongly affected by random or spatial differences in identity of newly colonizing taxa, which might explain the observed initial variation among grasslands, while compositions might converge in later years with the arrival of additional species.

Successional changes with respect to both the identity and the number of taxa can also be related to the development of a vertical soil profile. In a study of secondary succession of wheat fields into beech wood, Scheu and Schulz (1996) for instance showed that in early successional stages oribatids predominantly colonized the litter layer, while in more developed beech woods the upper mineral soil was also colonized and actually more taxa were present in this soil layer. Finally, the stabilization of

the taxon richness in combination with a continued succession in species composition may indicate that some mites species remain present in very low numbers in arable land, and increase in numbers when biotic and abiotic circumstances in grassland at different stages of succession have become favorable. More intense sampling at different spatial scales in arable lands may help to assess whether such “latent” populations of mites do indeed exist in arable lands. Well known abiotic changes in aging grasslands are e.g.,: a slow build-up of soil organic matter, an increase in total nitrogen, a decrease of the pH, and a loss of phosphate (Van der Wal et al., 2006; Wissuwa et al., 2013). Inverse patterns in abiotic parameters have been observed after grassland was converted to arable land (Postma-Blaauw et al., 2012).

A detailed study of the functional diversity in soil organisms will allow a better understanding and better modeling of the interactions between management or environmental factors and ecosystem services (Duru et al., 2015). When studying functional diversity in terms of feeding guilds, our observations at Lusignan suggested that all major guilds were present even in intensively managed arable soils, despite yearly tillage and high nitrogen inputs. Grassland soils, however, showed much higher biomass of fungivore, herbivore and especially predatory mites. The



same guilds also responded in a significant way to time after conversion in the Veluwe chronosequence experiment, but the timing of the responses differed between guilds and between the biomass and taxon richness of these guilds. The observed patterns suggest that predatory mites are able to develop high abundances within 10 years after a conversion from arable land to grassland and that abundances are relatively stable afterwards, while additional taxa arrive with time, resulting in a steady increase in number of taxa. This is in contrast with the patterns observed for herbivores, of which the number of taxa stabilized already early in succession, while the abundances of the species kept increasing over time. The community of decomposers seems to develop relatively slowly both in terms of abundance and taxon richness. Herbofungivorous and fungivorous grazers typically are almost completely absent from high-input grasslands, and appear in high numbers in low-input grassland (Siepel, 1996). Many species in these groups have low population growth rates and low dispersal rates (Siepel, 1991, 1994), which might explain the observed slow increases in abundance and taxon richness.

Implications of Our Results for Ecosystem Service Provision

Additional studies, preferably using e.g., reciprocal transplantation and inoculation experiments to test which species arrive and survive under various conditions (e.g., Klimek and Rolbieki, 2014), will be needed to assess exactly which factors drive the observed differences in mite community composition among our study fields. Yet, when combining the results of the Lusignan and Veluwe experiments, we can deduct a few general trends in the abundance and taxon richness of particular mite

TABLE 3 | Results of Linear Mixed Models for the Veluwe LTO chronosequence, testing for the effect of time since conversion from arable land to grassland on mite biomass (per plot; $N = 21$) and taxon richness (per site; $N = 9$) in the total community as well as per feeding guild.

Variable	Group	df	F	P
Biomass	All mites	1	12.338	0.010
	Bacterivores	1	0.120	0.739
	All fungivores	1	4.612	0.069
	Fungivore grazers	1	14.432	0.001
	Fungivore browsers	1	0.405	0.545
	All herbofungivores	1	2.861	0.135
	Herbofungivore grazers	1	1.973	0.203
	Opp. herbofungivores	1	1.633	0.242
	All herbivores	1	12.042	0.010
	Herbivore grazers	1	9.322	0.018
	Herbivore browsers	1	0.793	0.403
	All predators	1	4.425	0.073
	General predators	1	4.665	0.068
	Arthropod predators	1	1.647	0.240
	Nematode predators	1	2.795	0.107
	Omnivores	1	1.445	0.241
	Main decomposers	1	8.195	0.024
Taxon richness	Total	1	14.367	0.007
	Bacterivores	1	0.513	0.497
	Fungivores	1	4.608	0.069
	Herbofungivores	1	3.120	0.121
	Herbivores	1	4.190	0.080
	Predators	1	3.138	0.001
	Omnivores	1	1.034	0.319
	Main decomposers	1	14.813	0.006

Degrees of freedom (df), F-Value (F), and test significance (P) are given for each model. Significant effects ($P < 0.05$) are given in bold text.

feeding guilds during early and late succession after conversion from arable land to grassland. Using relations known from the literature (as outlined in the introduction), we can then derive hypotheses on the implications this may have for the ecosystem services of pest regulation, nutrient cycling and soil formation.

After conversion to grassland the biomass of predatory mites showed a rapid increase, while the number of taxa increased markedly in the first years, and continued to rise until later years. The limited number of predatory species in arable land may have been the result of a similarly low diversity of prey species and a few predatory species may still have a strong suppressing effect if abundant and selectively preying on a particular pest species (Straub, 2006). Yet, one can more generally expect that a higher taxonomic richness of predators would result in a higher potential to control a wide variety of pest species (Snyder and Straub, 2005) and thus a higher potential to suppress newly invading pests. In that sense, the potential for pest regulation in arable land was supposedly at a relatively low level, and developed quickly to higher levels in grassland, where it continued to slowly increase with time.

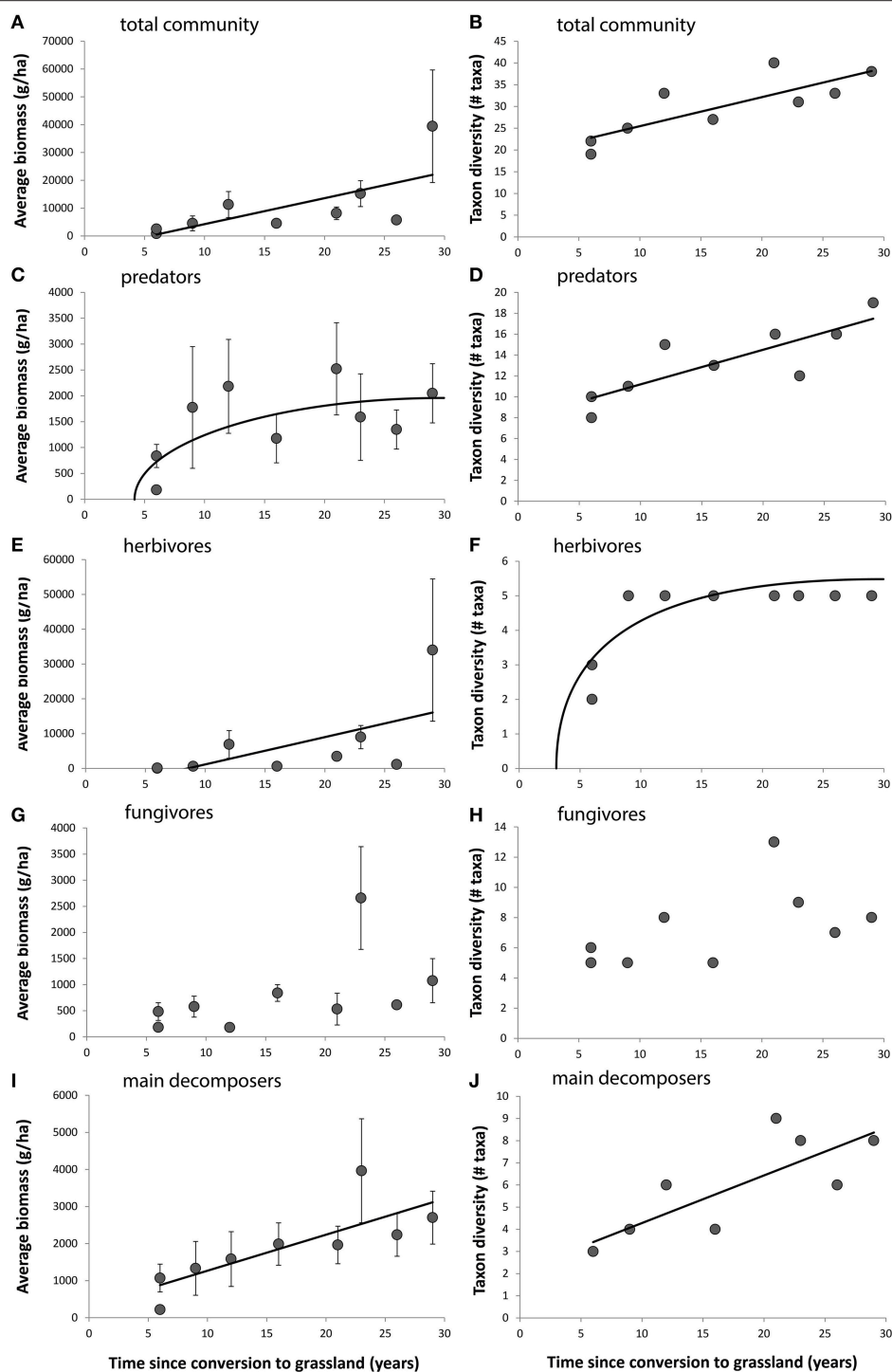


FIGURE 5 | Relations for the Veluwe LTO chronosequence, between either average biomass (A,C,E,G,I) or taxon richness (B,D,F,H,J) per field and time since conversion to grassland. Error bars represent standard errors. Separate relations are shown for the total mite community (A,B), the predators (C,D), the herbivores (E,F), the fungivores (G,H) and the main decomposers (I,J).

Soil formation depends on a broad palette of burrowing activities, fragmentation of organic material, the leaving behind of the cuticle of prey items, the grazing or browsing effects on fungal hyphae, and the production of feces (e.g., Van Vliet and

Hendrix, 2007). Because of the variety in processes involved, the concerted impacts of all the different mite species can likely best be represented by the total mite biomass. After conversion from arable land to grassland, the total mite biomass increased both

on the short term (Lusignan) and on the long term (Veluwe). This indicates that the general contribution of soil mites to soil formation may be expected to increase over a long period after arable land has been converted to grassland.

Upon conversion to grassland, the biomasses of fungivores, herbivore grazers and main decomposers increased, both in the Lusignan rotation and in the Veluwe chronosequence. As the main decomposers feed on fungi or on plant roots, an increase in the biomass of these groups can be expected in environments with a thick organic layer and/or a high root biomass (Luxton, 1982; Maraun and Scheu, 2000). In turn, high root biomass and high microarthropod biomass are associated with an increase in the mass flows through different channels of the soil food web. These food web interactions are too complex to accurately predict the outcome for overall decomposition and soil organic matter regulation, but some impact cannot be excluded (Siepel and Maaskamp, 1994) and therefore consequences can be expected for all ecosystem services that are associated with soil organic matter. In fact, nearly all soil ecosystem services may be affected, as their provision is associated with soil organic matter.

In both field studies, the soil mites of groups that are relevant for pest regulation, nutrient cycling and soil formation generally increased after the conversion of arable land to grassland. Such results suggest that with increasing age, the soil mite community of extensive grasslands will develop an increasing capacity of performing these ecosystem functions and services. Yet, here we did not directly test the provisioning of these services at the time of sampling. Targeted experiments, testing e.g., biological control potential or decomposition rates in soils in the presence of artificially created mite communities (e.g., Couteaux et al., 1991), will be needed to test the hypotheses raised in this study (Adhikari and Hartemink, 2016). Given the previous results by e.g., Horrocks et al. (2014) showing a potentially long time lag in changes in service provision after a land use change (as well as our biotic data suggesting differences in speed of establishment among functional groups), such experiments should ideally run for equivalently long periods of time.

For grasslands under conservation management, the later stage development of soil mite communities may be accelerated by species introductions through application of topsoil and sods from late-successional stages, in order to optimize the above discussed functions and services. The relevance thereof may, however, change with the converted land use. Thus, pest control as an ecosystem service may be less important for grasslands than it is for arable cropping systems, decreasing in relevance and value with decreasing agricultural use. Likewise, the ecosystem function of nutrient cycling is valued for natural soil fertility in the cropping system, whilst the opposite (nutrient conservation and even immobilization) becomes more desirable under grassland conservation management.

As the development of the soil mite community is boosted upon conversion toward grassland, 1 year or several years of grassland in extended crop rotations may already enhance the provision of these ecosystem services. A conversion back to arable land will likely result in similarly rapid change in mite

communities, altering the species composition and reducing both the biomass and number of taxa of the local mite community (e.g., Postma-Blaauw et al., 2012). However, preliminary results for an additional land use treatment at Lusignan, consisting of an 3 years grassland: 3 years arable land rotation, suggest that even after a conversion back to arable land at least part of the diversity is maintained albeit with a much lower biomass (De Groot et al., unpublished data). Thus, for sustainable arable cropping systems aiming at optimizing the provision of ecosystem services it could be worthwhile to widen the rotation to include a certain period of grassland. Longer periods of grassland in the rotation cycle might be preferable, since our chronosequence data suggest that decades of extensive management may be required to reach the full potential to which mites may contribute to service provisioning. Further research, studying changes in mite communities over time in soils subjected to different grassland / arable land rotation schemes, are required to get a full insight in how periods of grassland can be incorporated in crop rotation schemes in such a way that the services contributed by soil mites are maximized. This understanding will help to evaluate and further develop green growth rotation schemes and maintaining permanent grassland as greening practices stimulated under the EU Common Agriculture Policy.

AUTHOR CONTRIBUTIONS

WD and JF performed the field work, with assistance from XC. WD performed the species identifications and biomass calculations. GD and GJ analyzed the data. GD, GJ, and JF wrote the first version of the manuscript, with input from WD and XC.

FUNDING

This research was funded by the European Commission through the FP7 project EcoFINDERS (Ecological Function and Biodiversity Indicators in European Soils; www.ecofinders.eu; grant number FP7-264465) and by the strategic research program KBIV “Sustainable spatial development of ecosystems, landscapes, seas and regions” which is funded by the Dutch Ministry of Economic Affairs and carried out by Wageningen University and Research Centre.

ACKNOWLEDGMENTS

We thank the site managers of the two LTO sites (Veluwe: Prof. Wim van der Putten; SOERE ACBB Lusignan: Abad Chabbi) for providing the opportunity for research and for valuable details on the history of the sites. We thank Prof. Henk Siepel for his advice on a proper calculation of mite biomasses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fenvs.2016.00015>

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Conflict of Interest Statement: 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.

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Response of Soil Properties and Microbial Communities to Agriculture: Implications for Primary Productivity and Soil Health Indicators

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OPEN ACCESS

Edited by:

Karl Ritz,
University of Nottingham, UK

Reviewed by:

Jayanta Kumar Patra,
Yeungnam University, South Korea
Rajan Ghimire,
New Mexico State University, USA

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equally to this work.

Specialty section:

This article was submitted to
Agroecology and Land Use Systems,
a section of the journal
Frontiers in Plant Science

Received: 12 September 2015

Accepted: 22 June 2016

Published: 12 July 2016

Citation:

Trivedi P, Delgado-Baquerizo M,
Anderson IC and Singh BK (2016)
Response of Soil Properties
and Microbial Communities
to Agriculture: Implications for Primary
Productivity and Soil Health
Indicators. *Front. Plant Sci.* 7:990.
doi: 10.3389/fpls.2016.00990

Agricultural intensification is placing tremendous pressure on the soil's capacity to maintain its functions leading to large-scale ecosystem degradation and loss of productivity in the long term. Therefore, there is an urgent need to find early indicators of soil health degradation in response to agricultural management. In recent years, major advances in soil meta-genomic and spatial studies on microbial communities and community-level molecular characteristics can now be exploited as 'biomarker' indicators of ecosystem processes for monitoring and managing sustainable soil health under global change. However, a continental scale, cross biome approach assessing soil microbial communities and their functional potential to identify the unifying principles governing the susceptibility of soil biodiversity to land conversion is lacking. We conducted a meta-analysis from a dataset generated from 102 peer-reviewed publications as well as unpublished data to explore how properties directly linked to soil nutritional health (total C and N; C:N ratio), primary productivity (NPP) and microbial diversity and composition (relative abundance of major bacterial phyla determined by next generation sequencing techniques) are affected in response to agricultural management across the main biomes of Earth (arid, continental, temperate and tropical). In our analysis, we found strong statistical trends in the relative abundance of several bacterial phyla in agricultural (e.g., *Actinobacteria* and *Chloroflexi*) and natural (*Acidobacteria*, *Proteobacteria*, and *Cyanobacteria*) systems across all regions and these trends correlated well with many soil properties. However, main effects of agriculture on soil properties and productivity were biome-dependent. Our meta-analysis provides evidence on the predictable nature of the microbial community responses to vegetation type. This knowledge can be exploited in future for developing a new set of indicators for primary productivity and soil health.

Keywords: soil health, indicators, agriculture intensification, soil bacteria

INTRODUCTION

Soil health is the capacity of a soil to function, within natural or managed ecosystem boundaries, to sustain plant productivity, maintain water and air quality, support human well-being, and provide habitats for biodiversity (Doran and Zeiss, 2000; Doran, 2002; Gugino et al., 2009). Human impacts on soil health largely emerge from the need to meet the food, fiber, and fuel demands of an ever increasing population. In the last few decades significant efforts have been made to increase agricultural productivity through increased fertilization and pesticide application, improved irrigation, soil management regimes and crops, and massive land conversions (Tilman et al., 2002). There is increasing concern, however, that agricultural intensification is placing tremendous pressure on the soil's capacity to maintain its other functions leading to large-scale ecosystem degradation and loss of productivity in the long term (Tilman et al., 2001; Foley et al., 2005; Vitousek et al., 2009). For example, conversion of natural ecosystems to agricultural lands have incurred substantial environmental costs, including desertification, increased emissions of greenhouse gasses, decreased organic matter in soils, loss of biodiversity, and alterations to biogeochemical and hydrological cycles (Balmford et al., 2005). Modern agriculture thus faces great challenges not only in terms of ensuring global food security by increasing yields but also mitigating the environmental costs particularly in the context of a changing environment and growing competition for land, water, and energy (Chen et al., 2014). Therefore, there is an urgent need to find early indicators of soil health degradation in response to agricultural management (Grime, 1997; Cardoso et al., 2013).

Different terrestrial biomes may respond differentially to agricultural over-exploitation. For instance, arid lands, which occupy 40% of the globe and sustain 38% of the human population (Millennium Ecosystem Assessment [MEA], 2005), are very low productivity systems and contain low levels of nutrients (Reynolds et al., 2007; Feng and Fu, 2013). These ecosystems are highly vulnerable to global environmental changes and desertification (Reynolds et al., 2007; Dai, 2013) and may further suffer high reductions in nutrient availability in response to agricultural over-exploitation (Delgado-Baquerizo et al., 2013). On the other hand, highly productive agro-systems such as those from tropical regions may be highly resistance/resilience to agriculture uses, in part due to their rapid organic matter turnover and moisture/water availability (Schlesinger and Bernhardt, 2013). Limited effort has been made to understand the global trends that characterize microbial community composition in natural and agricultural systems (Crowther et al., 2014) which hinder our ability to anticipate the consequences of conversion in the different biomes on Earth.

Evaluation of soil health requires indicators of chemical, physical and biological (including microbial) components contribute to maintaining soil health. Cultivation is known to generally reduce the amount of soil organic matter thus reducing nutrient availability (Schlesinger and Bernhardt, 2013). Similarly, changes in land use are altering both microbial community structure and diversity in terrestrial ecosystems

(Rodrigues et al., 2013). Since soil bacterial communities drive many different ecosystem functions (e.g., Delgado-Baquerizo et al., 2016b), and their abundance, richness, and composition are sensitive to the changes in the land use and management (Gans et al., 2005; Wall et al., 2010; Singh et al., 2014), they have been considered as early indicators of change in the quality of soil ecosystems (Kennedy and Stubbs, 2006). In some instances, changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation (Pankhurst et al., 1997; Nielsen et al., 2002). At local scale fluctuations in microbial diversity and community composition are correlated with reductions in soil C and nitrogen (N) (Acosta-Martinez et al., 2008, 2010; Jangid et al., 2008; Trivedi et al., 2015). On global scale, however, land use change to agriculture systems on the soil C and N contents are more idiosyncratic (Johnson and Curtis, 2001), and negligible effect of conversion has been reported on microbial biomass from several biomes (Holden and Treseder, 2013). Since microorganisms are involved in many soil processes, they may also give an integrated measure of soil health, an aspect that cannot be obtained with physical/chemical measures alone (Nielsen et al., 2002; Kibblewhite et al., 2008; Mueller et al., 2010; Sharma et al., 2011). In recent years, major advances in soil meta-genomic and spatial studies on microbial communities and community-level molecular characteristics can now be exploited as 'biomarker' indicators of ecosystem processes for monitoring and managing sustainable soil health under global change. However, a continental scale, cross biome approach assessing soil microbial communities and their functional potential to identify the unifying principles governing the susceptibility of soil biodiversity to land conversion is lacking.

In the face of current anthropogenic pressure on soil ecosystems, for instance owing to agricultural intensification and climate change, there is a need to better understand the effects of these factors in order to predict and mitigate the impacts of such changes (Kuramae et al., 2012). However, reliable predictions of the potential consequences of perturbations are hampered by the lack of global level baseline knowledge on soil properties and soil microorganisms. Herein we conducted a meta-analysis to explore how soil properties (pH, total C and N; C:N ratio), primary productivity (NPP) and microbial diversity and composition (relative abundance of major bacterial phyla) are affected in response to agricultural management across the main biomes of Earth (arid, continental, temperate and tropical). The aim of the meta-analysis was to identify the impact of agriculture practices on soil nutritional health and microbial communities. We also aimed to examine if the response of microbial community to agriculture is consistent across all the biomes. We collected data from 102 peer-reviewed publications as well as unpublished data to create a global dataset of soil bacterial diversity and composition evaluated with next generation sequencing techniques (mostly 454 Pyrosequencing). Our meta-analysis revealed foreseeable nature of the microbial community responses to vegetation types suggesting that the microbial indicators can be developed

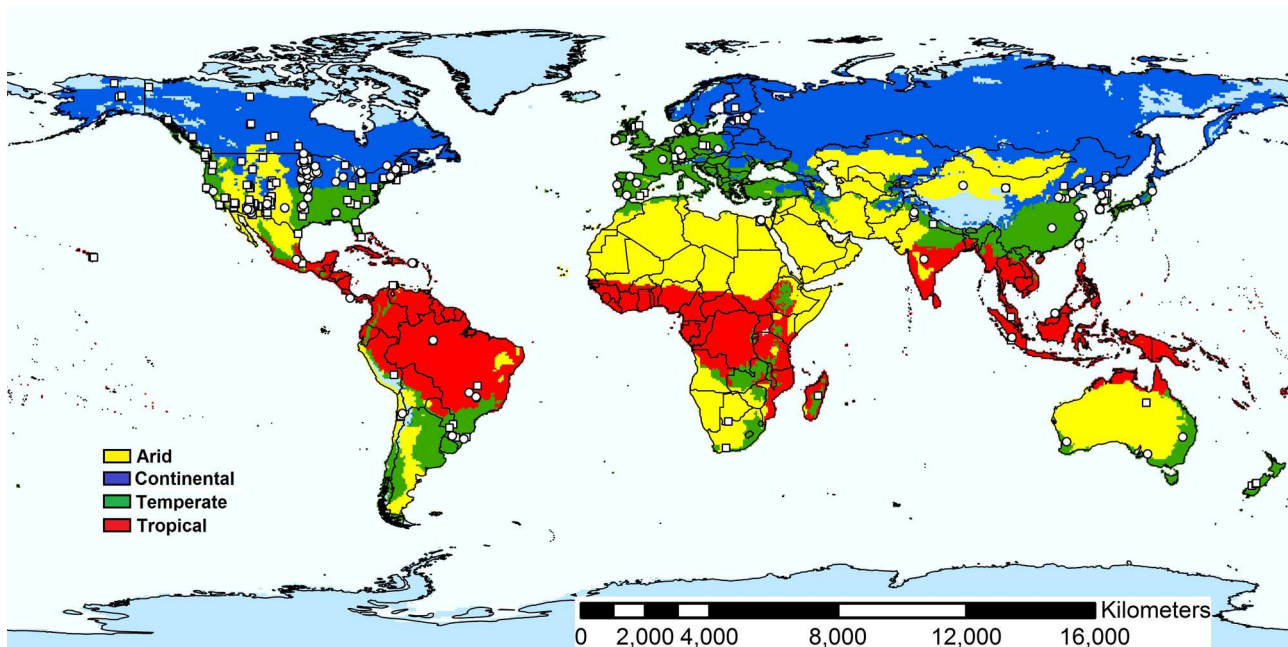


FIGURE 1 | Locations of the soil samples included in this study. Agricultural soil samples ($n = 165$) and natural soil samples ($n = 353$) are shown as circles and squares, respectively. The sites were selected based on a meta-analysis consisting of both published and unpublished data wherein bacterial diversity and compositions is described based on next generation sequencing techniques (either 454 or Miseq) from both. From those experimental studies that manipulated environmental conditions (e.g., nutrients or climatic conditions) we only used the data from the control treatment (See Material and Methods for more details).

as tools for prediction for primary productivity and soil health.

MATERIALS AND METHODS

Data Collection

We collected data on soil bacterial diversity based on next generation sequencing techniques (either 454 or Miseq) from both published and unpublished data. We first conducted a search using SCOPUS¹ (on September 2014). The following keyword combinations were used: (1) “bacterial community” AND “soil” AND “Pyrosequencing”; and (2) “bacterial community” AND “soil” AND “Illumina.” We found ~300 references. Within these references, studies were chosen for inclusion in our analyses only if they met the following criteria: (1) were carried out in the field in terrestrial ecosystems, (2) contained the spatial location where they were carried out (latitude and longitude), as well as data on soil total C and pH; (3) provided information on Shannon bacterial diversity at 97% of similarity; (4) included data on the relative abundance of soil bacterial phyla, (5) used next generation sequencing techniques (either 454 or Miseq) and (6) were located in arid, continental, temperate or tropical ecosystems (Koppen classification; Kottek et al., 2006). From those experimental studies that manipulated environmental conditions (e.g., nutrients or climatic conditions) we only used the data from the

control treatment. The dataset included geographical locations covering all continents and biomes where agriculture is in practice (Figure 1; Data Sheet S1).

Technical information related to this study (e.g., primer sets, sequencing technology) and validation of the impact of various approaches to generate data on the conclusions is largely discussed in Delgado-Baquerizo et al. (2016a).

Microbial Related Parameters

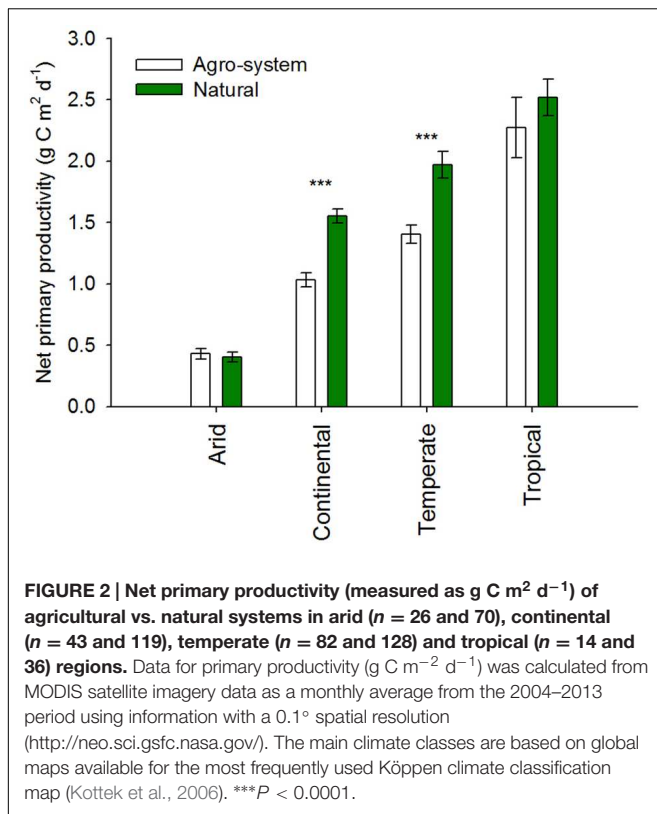
In total of 102 articles containing data on Shannon bacterial diversity and bacterial community composition (relative abundance of major groups) were obtained from our literature search (Data Sheet S1 for complete list of studies). We completed this database with data from the National Ecological Observatory Network (NEON; 24 soil samples) in the United States of America², Canadian MetaMicroBiome Library³ (11 soil samples) and with unpublished data from sites in Australia (12 soil samples) and Scotland (6 soil samples) (data available from authors). From our meta-analysis we obtained a total of 518 independent soil samples (353 and 165 soil samples belong to natural and agricultural systems, respectively). Bacterial Shannon diversity and composition was available for 61 and 100% of these sites, respectively.

For all the samples available, we gathered data on the relative abundance of the following major bacteria phyla: *Proteobacteria*,

¹<http://www.scopus.com/>

²<http://www.neoninc.org/>

³www.cm2bl.org



Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadete, Plantomycetes.

Soil Properties and Net Primary Productivity (NPP)

We collected information on the following soil properties: soil total C, total N, C:N ratio and pH from the studies selected for meta-analysis. Most of the studies in our meta-analysis used elemental CNH analyzer for the estimation of soil C. This method analyzes both inorganic and organic carbon hence can overestimate the amount of SOC in the samples. Data for NPP (g C m⁻² d⁻¹) was calculated from MODIS satellite imagery data as a monthly average from the 2004 to 2013 period using information with a 0.1° spatial resolution⁴.

Ecosystem Classification

We determined the main climate classes in each of the study sites based on global maps available for the most frequently used Köppen climate classification map (Kottek et al., 2006): A (tropical), B (arid), C (temperate), and D (continental). We completed climate gaps in our dataset using local and regional database. These analyses were done with ESRI ArcGIS Desktop 10.

Statistical Analyses

We used two-way ANOVAs to evaluate changes in NPP, soil properties and microbial community features (main bacterial phyla and Shannon diversity) among different biomes (arid, temperate, continental and tropical) and land use type (agricultural vs. natural). Biomes and land use type were included as fixed factors in these analyses. These statistical analyses were carried out using IBM SPSS 15.0 (SPSS Inc, Chicago, IL, USA). We also used Spearman correlation analyses to evaluate the relationship between microbial community features (main bacterial phyla and Shannon diversity) with soil properties and NPP.

RESULTS

NPP and Soil Properties of Agricultural vs. Natural Soils in Different Regions

Net primary productivity (measured as g C m⁻² d⁻¹) was significantly higher in natural as compared to agro-systems from continental and temperate regions ($P < 0.0001$) (Figure 2). However, our meta-analysis did not reveal a significant difference between agricultural and natural ecosystems for arid and tropical regions (Figure 2). pH of agriculture soils from continental, temperate, and tropical regions were higher than natural soils (Figure 3A). The data revealed an approximate increase of 1.5, 1.0 and 0.5 units in soil pH from agriculture soils as compared to natural soils in continental ($P < 0.0001$), temperate ($P < 0.0001$) and tropical ($P < 0.01$) regions, respectively. There was no difference in the pH values associated with agricultural vs. natural soils in arid regions.

Soil % C in natural soils from temperate regions was approximately 8.0% and this was significantly higher ($P < 0.01$) than the agricultural soils (~5.5%, Figure 3B). The data revealed no significant difference in the % of total C in agricultural vs. natural soils from continental and tropical regions. In arid regions, our meta-analysis revealed significantly higher ($P < 0.01$) % of total C in agricultural as compared with natural soils. In arid regions the % of total C was approximately 2.5 and 1.7% for agricultural and natural soils, respectively.

Our analysis revealed variability in the soil N content when comparing agricultural vs. natural soils from different regions. In arid regions, the total N % in agricultural soils was approximately 0.22% which was significantly higher ($P < 0.001$) than natural soils (~0.15%, Figure 3C). We observed no significant trends in % of soil N in agricultural vs. natural soils in the other three regions. However, agricultural soils from continental regions had a higher N content compared to natural soils while the opposite trend was observed in temperate regions. The % N of agricultural and natural soils was similar in tropical regions.

As an average, arid and temperate regions showed the lowest and highest C:N ratio in this study, respectively (Figure 3D). The C:N ratio of agricultural soils from arid and tropical regions was higher ($p < 0.01$) when compared to natural soils from similar regions. In contrast, the C:N ratio of natural soils was significantly higher ($P < 0.0001$) than agricultural soils in temperate regions.

⁴<http://neo.sci.gsfc.nasa.gov/>

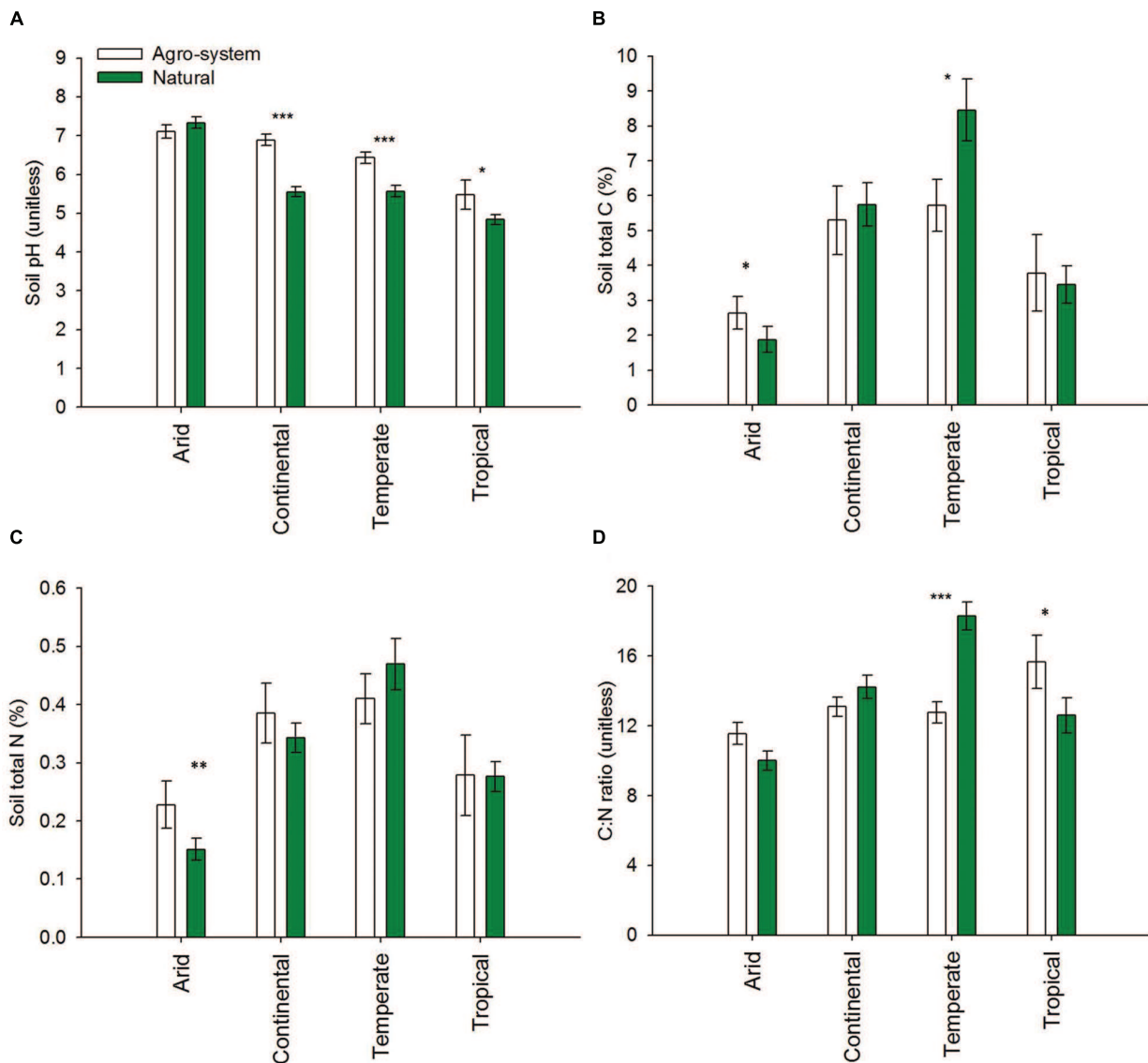


FIGURE 3 | Soil chemical properties [(A) Soil pH; (B) Soil total C (%); (C) Soil total N (%); and (D) C/N ratio] of agricultural vs. natural systems in arid ($n = 26$ and 70), continental ($n = 43$ and 119), temperate ($n = 82$ and 128) and tropical ($n = 14$ and 36) regions. The sites were selected based on a meta-analysis consisting of both published and unpublished data wherein bacterial diversity and compositions is described based on next generation sequencing techniques (either 454 or Miseq; see Material and Methods for more details). The main climate classes are based on global maps available for the most frequently used Köppen climate classification map (Kottek et al., 2006). * $P < 0.01$; ** $P < 0.001$; * $P < 0.0001$.**

In continental regions there was no significant difference in the C:N ratio from agricultural and natural soils.

Microbial Data

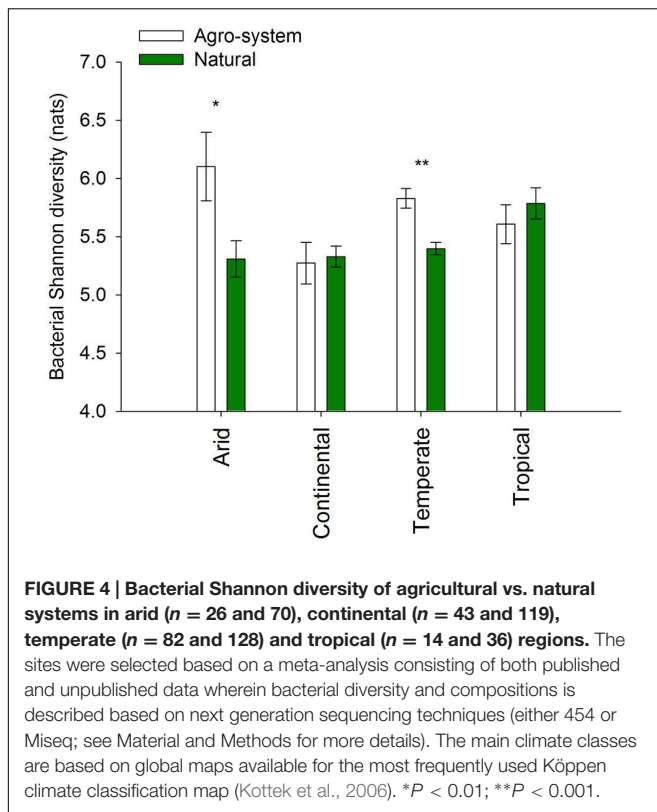
Microbial Diversity in Agricultural vs. Natural Systems in Different Regions

We selected the Shannon diversity index as our metric of alpha diversity because it is highly recommended and commonly used when analyzing microbial diversity (He et al., 2013), and has been shown to reduce the bias in relation to other diversity metrics, such as the number of OTUs, when comparing data from multiple sources (He et al., 2013). Our analysis revealed

significantly higher microbial diversity in agricultural systems as compared to natural systems in arid ($P < 0.01$) and temperate ($P < 0.001$) regions (Figure 4). Microbial diversity was lower in agricultural systems in continental and tropical regions as compared with natural systems; however, the trends were not statistically significant.

Relative Abundance of Bacterial Phyla in Agricultural vs. Natural Soils in Different Regions

The relative abundance of major bacterial phyla in agricultural and natural soils from different regions is presented in Figure 5. The relative abundance of *Acidobacteria* was significantly



greater in natural soils as compared to agricultural soils from arid ($P < 0.001$), continental ($P < 0.0001$), and temperate ($P < 0.0001$) regions. In arid regions, the relative abundance of *Acidobacteria* was nearly three times greater in natural soils as compared to agricultural soils. Similar to other regions, our meta-analysis showed higher relative abundance of *Acidobacteria* in natural vs. agricultural soils in tropical regions, however, this was not statistically significant. Our meta-analysis revealed higher relative abundance of phylum *Proteobacteria* in natural soils as compared to agriculture soils from all the studied regions. This trend was significant in soil from continental ($P < 0.01$), temperate ($P < 0.0001$), and tropical regions ($P < 0.01$). Our analysis further revealed significantly higher relative abundance of *Cyanobacteria* in natural soils vs. agricultural soils from arid ($P < 0.0001$), continental ($P < 0.01$), and temperate ($P < 0.01$) regions. Interestingly in arid regions the relative abundance of this group was approximately 6 fold higher in natural as compared to agricultural soils.

In contrast we observed higher relative abundance of *Chloroflexi* in agricultural soils as compared to natural soils across all regions. The relative abundance of this phylum was 2 and 6 fold higher in agriculture soils as compared to natural soils from arid ($P < 0.001$) and temperate regions ($P < 0.0001$), respectively.

The relative abundance of phylum *Actinobacteria* was significantly higher in natural vs. agricultural soils from continental regions while an opposite trend was observed in soils from tropical regions. Our analysis did not show significant differences in the relative abundance of this phylum when

agriculture and natural soils were compared from arid and temperate regions. *Firmicutes* showed significant differences among agricultural and natural soils only in arid regions ($P < 0.0001$) where the relative abundance was approximately 5 fold higher in agricultural soils. The members of phylum *Verrucomicrobia* were significantly more abundant in agricultural soils as compared to natural soils from continental ($P < 0.0001$) and tropical ($P < 0.01$) regions while the relative abundance of *Planctomycetes* was significantly higher in natural soils compared to agricultural soils ($P < 0.01$) from continental regions (Figure 5).

Correlations between the NPP and Soil Properties with the Relative Abundance of Different Bacterial Phyla Across Different Regions

The correlations between the NPP and soil properties with the microbial data are presented in Supplementary Table S1. Bacterial diversity of the arid regions was significantly correlated with NPP ($\rho = 0.520$; $P < 0.001$). However, in our analysis we did not observe the same correlation in other regions. In arid regions, the relative abundance of phylum *Chloroflexi* ($\rho = -0.213$; $P = 0.037$) and *Proteobacteria* ($\rho = 0.283$; $P < 0.005$) was correlated with NPP. The relative abundance of phylum *Cyanobacteria* was negatively correlated with total soil C ($\rho = 0.206$; $P < 0.044$) and the C:N ratio of arid regions.

Our analysis showed an increase in the number of correlations between the relative abundance of various bacterial phyla, NPP and soil properties in continental and temperate regions. For example, in continental regions significant correlations were observed between the NPP and the relative abundance of *Acidobacteria* ($\rho = 0.347$; $P < 0.001$), *Actinobacteria* ($\rho = -0.190$; $P = 0.016$), *Chloroflexi* ($\rho = -0.276$; $P < 0.001$), and *Firmicutes* ($\rho = 0.336$; $P < 0.001$). In temperate regions, NPP and soils properties were correlated with the relative abundance of many different bacterial phyla. For example, in temperate regions NPP was significantly correlated with the relative abundance of all bacterial phyla except for *Planctomycetes*. Soil C was correlated with the relative abundance of *Acidobacteria*, *Firmicutes*, *Verrucomicrobia*, and *Proteobacteria*. In fact, in temperate regions the relative abundance of phylum *Acidobacteria*, *Firmicutes*, and *Proteobacteria* was significantly correlated with NPP and all soil properties analyzed in this meta-analysis. In tropical regions, NPP was correlated with the abundance of *Chloroflexi* ($\rho = 0.346$; $P = 0.014$) and *Verrucomicrobia* ($\rho = 0.294$; $P = 0.038$). With respect to this region, the data showed no significant correlation between total soil C and the relative abundance of different bacterial phyla.

DISCUSSION

NPP Differed between Agricultural and Natural Systems Only in Continental and Temperate Biomes

Terrestrial NPP represents the total annual growth of land vegetation and is the basic resource for food, fiber, and energy (Vitousek et al., 1986; Running, 2012; Krausmann et al., 2013). In

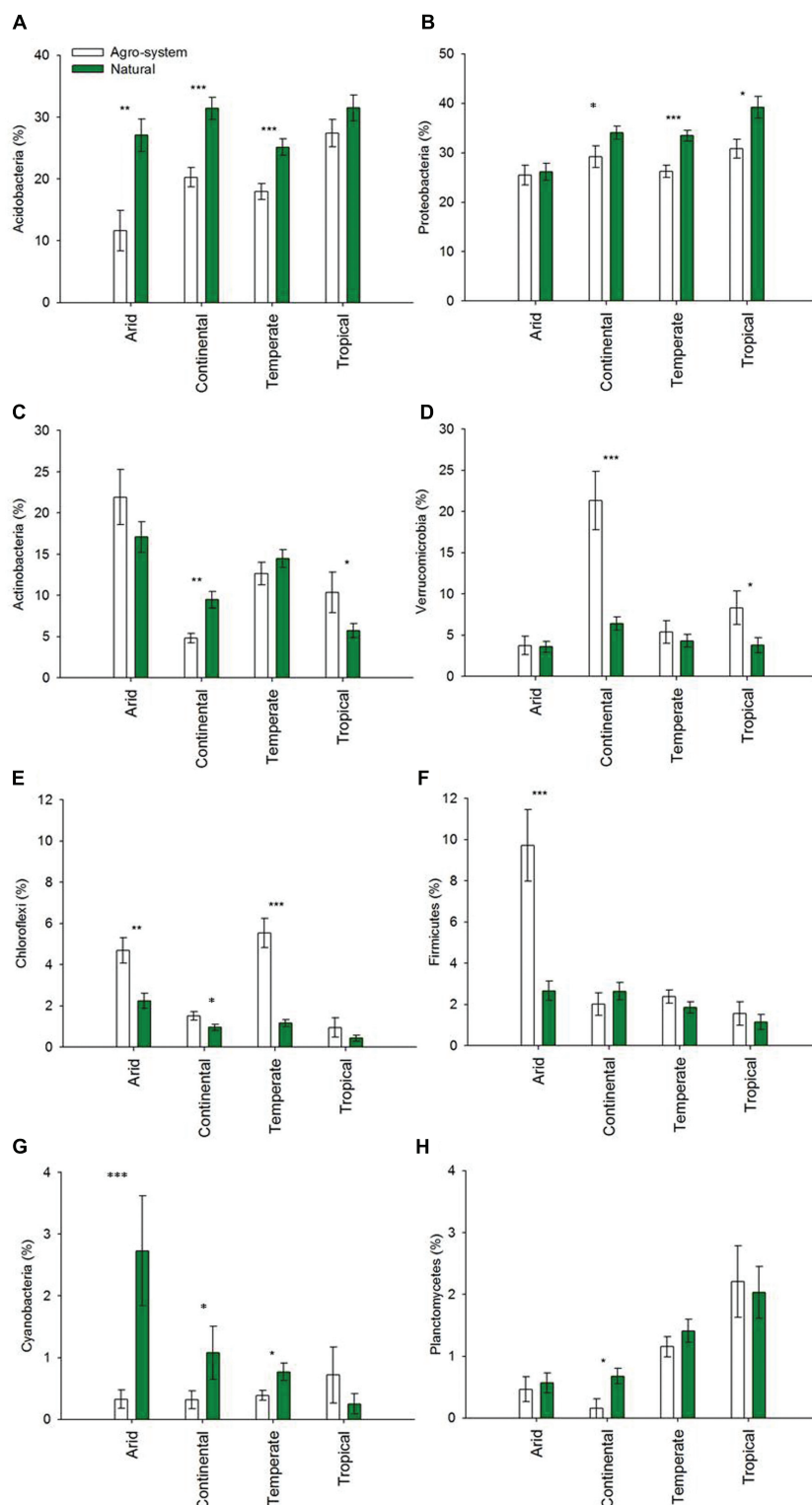


FIGURE 5 | Relative abundance of major bacterial phylum [(A) *Acidobacteria*; (B) *Proteobacteria*; (C) *Actinobacteria*; (D) *Verrucomicrobia*; (E) *Chloroflexi*; (F) *Firmicutes*; (G) *Cyanobacteria*; and (H) *Planctomycetes*] in agricultural vs. natural systems in different regions. The sites were selected based on a meta-analysis consisting of both published and unpublished data wherein bacterial diversity and compositions is described based on next generation sequencing techniques (either 454 or Miseq; see Material and Methods for more details). The main climate classes are based on global maps available for the most frequently used Köppen climate classification map (Kottek et al., 2006). * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

addition, terrestrial NPP is also a major component of the global C cycle, and a critical precursor to net C storage. Changes in NPP due to agricultural conversion could result in either enhancing or mitigating increments in atmospheric CO₂ concentrations and climate warming (Fargione et al., 2008; Searchinger et al., 2008). Latitudinal control of insolation (solar radiation that reaches earth surface) on photosynthesis results in a noticeable decrease in NPP from tropical ecosystems to those in the middle or higher latitudes (**Figure 2**). It is generally assumed that agricultural ecosystems are significantly less productive (e.g., by harvest-induced reductions in growing season length) than natural systems in the same environment (Smith et al., 2014). On the contrary it can also be argued that agricultural conversion at a local scale can increase NPP (e.g., by management inputs that reduce biophysical growth limitations) (Long et al., 2006). In our analysis we observed a significant reduction in NPP in agro-ecosystems as compared to natural ecosystems in continental and temperate environments (**Figure 2**). In similar environments, Smith et al. (2014) have reported a significant decrease in NPP due to agricultural conversion that was independent of conversion type, management intensity, crop type, or regions. Our analysis revealed a decrease in NPP in agro-ecosystems in tropical regions (**Figure 2**), however it was not as steep as reported by other workers (Smith et al., 2014). As most of our sites in tropical regions were situated in the industrialized west and Asia, the non-significant decrease in NPP in agricultural sites might be due to the relatively intensive management practices and crop types which could contribute to higher rates of productivity that more closely match those of natural vegetation (Gelfand et al., 2013; Smith et al., 2014). Similarly, in arid regions our analysis showed no differences between NPP of agricultural and natural systems (**Figure 2**). It seems that in nutrient poor systems, such as arid system climate constraints do not allow an increase in NPP. In arid regions, water availability will be the major constraint on NPP and the plants will be more sensitive to precipitation variation than soil management (Zhu and Southworth, 2013).

Trends Obtained from Properties Linked to Soil Nutritional Health Were Not Consistent in Agriculture vs. Natural Systems among All the Climatic Regions

Agriculture practices generally results in a decline in soil nutrients. However, nutrients inputs, from both natural and synthetic sources can improve plant growth that increases organic matter returns leading to improvement in soil quality (Smith et al., 2015). Changes in soil properties can vary markedly with type of land cover, climate, and method, extent of vegetation removal (e.g., land clearing, fires, mechanical harvest), and management post harvests. Here we discuss trends obtained from our meta-analysis on the soil chemical properties of agricultural vs. natural systems in different climatic regions.

Soil Carbon

As the dominant land-use change during the past century, conversion of natural systems for agricultural production has

greatly altered soil C dynamics at ecosystem, regional, and global scales (Foley et al., 2005; Bala et al., 2007; Don et al., 2011; Yonekura et al., 2012; Zhang et al., 2015). The depletion of soil total C due to the intensification of agriculture and land-use change from natural to croplands is exacerbated through agricultural practices with low return of organic material and other various factors including oxidation/mineralization, leaching and erosion (Post and Kwon, 2000; Wu et al., 2003; Lal, 2004; Zhang et al., 2015). In a meta-analysis, Guo and Gifford (2002) showed that the conversion of native forests and pastures to croplands reduced soil C stocks by 42 and 59%, respectively. The results varied, however, depending on factors such as annual precipitation, plant species and, the length of study periods. Our analysis indicated that total C % of agricultural soils were lower as compared to natural soils in temperate regions (**Figure 3B**). However, no significant difference in total C % in agricultural vs. natural systems were observed in other regions.

Previous studies have reported negative, positive, and negligible effects of land conversion on soil C content (Bashkin and Binkley, 1998; Vesterdal et al., 2002; Yang et al., 2011; Zhang et al., 2015). For example, 13% of the croplands included in a meta-analysis on the impact of tropical land use change on soil organic matter reported similar to higher soil C stocks in agricultural soils than forests (Don et al., 2011). The different sampling schemes, estimation methods, and the complexity of factors affecting soil C dynamics are attributed to the inconsistency in various studies (Don et al., 2011; Li et al., 2012). Following the land-use change, litter input from new vegetation will be terminated and replaced by litter from new vegetation, while the soil C derived from the former litter would be decomposed and mineralized by soil microbes (Zhang et al., 2013). Thus soil C stocks would be controlled not only by the degradation of old C (soil C previous to conversion) but also by the addition of new soil C (C derived from new vegetation after land use) (Del Galdo et al., 2003; Mendez-Millan et al., 2014). Our observations, particularly in Continental and Tropical regions, is in contrast to most previous studies (Guo and Gifford, 2002; Don et al., 2011) that have reported significant lower soil C in agricultural soil as compared to natural soils. This discrepancy may arise due to differences in management practices and disturbance regimes including tillage, residue retention, grazing and the duration of change in land use. Wiesmeier et al. (2015) has reported that soil cultivation may not generally result in the strong decline in soil C content, as management practices such as tillage probably promote the formation of organo-mineral associations and relocation of soil C with depth may decrease decomposition. No significant change in soil C in agricultural soils in arid/tropical regions results from boosted productivity and higher turnover rates adding more C to the soil due to organic manure/fertilizer application as well as the effect of crop residue, and irrigation regimes (Zhang et al., 2013).

Soil N

Conversion of natural lands into arable lands is not only characterized by losses of ecosystem C stocks, but also by significant losses of ecosystem N stocks along hydrological pathways, gaseous volatilization or through erosion (Tiessen

et al., 1982; McLauchlan, 2006). A meta-analysis using mainly data from tropical sites indicated that the average loss of soil N after conversion of forests to croplands was 15% (Murty et al., 2002). Dalal et al. (2013) reported that conversion of native vegetation to perennial pasture and cropland in Australia resulted in N losses of more than 20 and 38%, respectively. Our analysis did not reveal significant differences in soil N % between agricultural and natural soils from continental, temperate, and tropical regions. We argue that the extensive use of chemical N fertilizer in agricultural soils will compensate for N losses through natural processes thereby maintaining total soil N concentrations to the levels similar to natural soils. In addition, the introduction of leguminous plants to crop rotations (Tiessen et al., 1982) or the application of organic fertilizers (Griffin et al., 2005), can support an increase in N stocks. Our analysis showed a significant higher total soil N in agricultural systems from arid regions compared to natural systems. In arid regions SOC and N stocks have been reported to depend strongly on soil types with strong interactions between soil type and land use (Mayes et al., 2014). Increases in the soil N in arid regions might also be the result of preference to grow leguminous crops which have a lower water requirement (Creswell and Martin, 1998).

Soil pH

Comparing soils from a similar climate in tropical, continental, and temperate regions, soils from agricultural systems tend to be more alkaline than natural soils. Liming in agricultural soils is also one of the major factors leading to an increase in soil pH (Armstrong et al., 2015). The greatest (positive) effects with pH were seen in the acidic soils, however, in arid regions where the pH tends to be more alkaline, our analysis showed no significant differences between agricultural vs. natural soils suggesting that impact of agricultural practices was soil dependent. Contrary to agricultural systems, natural ecosystems tend to be more acid in general. This difference in acidity can be generated through several mechanisms, including increased production of organic acids or through the generation of carbonic acid from higher rates of autotrophic respiration in natural soils (Richter and Markewitz, 1995). The increased acidity of forests may also be caused by increased uptake of cations by trees and consequent changes in the proportions of cations adsorbed to the soil exchange complex (Jobbagy and Jackson, 2004). Berthrong et al. (2009) have reported that higher acidity in natural soils can also be driven by changes in the proportions of cations such as Ca, Mg, Na, and K.

Response of Soil Bacterial Community

It could be argued that our analyses suffer from biases such as those related to the different primer sets used by the studies included in our database. However, results from our previous study (Delgado-Baquerizo et al., 2016b) clearly demonstrate that primers pairs, sequencing platform, and the method of soil sampling does not significantly alter the microbial diversity and relative abundance of major soil bacterial phyla and that next generation sequencing data can be as useful as other

available data to evaluate global patterns in microbial ecology. We argue that the point of variability in the results on the relative abundance of major bacterial phyla using different primer-set (Engelbrektson et al., 2010; Cai et al., 2013; Fredriksson et al., 2013) can be critical in local studies as in these cases the variability among primer sets may overlap the spatial variability within a particular plot or the effects of a given treatment on the abundance of these bacteria. However, small changes in relative abundance of different phyla that could be attributed to using different primer sets (Engelbrektson et al., 2010; Cai et al., 2013; Fredriksson et al., 2013) is unlikely to bias results from a global-scale meta-analysis like the one performed in the present study (Delgado-Baquerizo et al., 2016b). These variations are indeed a part of the intrinsic noise one may expect in similar meta-analyses conducted with other soil microbial variables (Fierer et al., 2009; Serna-Chavez et al., 2013).

Microbial Diversity in Agriculture vs. Natural Systems

Understanding the mechanisms that control the extent to which soil properties and microbial communities change following the conversion of natural to agricultural systems is of paramount importance to comprehend the consequences of land use changes for soil health and agricultural productivity (Sala et al., 2000). Management practices such as tillage and crop rotation; periodic fertilization; and pesticide application generate temporal and spatial changes in soil physical and chemical properties in agricultural systems (Carbonetto et al., 2014). The agricultural systems thus represents rapidly fluctuating environments with highly variable resource gradients and greater bio-physical and chemical heterogeneity as compared to natural systems, thereby providing a wide range of niches for microbial growth. This variability and heterogeneity can result in increased diversity in agricultural systems as compared to stable natural systems. In fact our meta-analysis revealed that microbial diversity increased significantly in agricultural systems of arid and temperate regions (**Figure 4**). The fact that diversity increased or was not markedly altered (continental and tropical regions) as a consequence of agriculture activities is not unexpected. In fact, microbial communities in natural systems may be limited by nutrient availability and therefore fertilizer addition may allow colonization by new species from the regional pools (Jangid et al., 2008; Upchurch et al., 2008; Jesus et al., 2009; Lee-Cruz et al., 2013; Crowther et al., 2014; Figuerola et al., 2015). However it has been reported that although local taxonomic and phylogenetic diversity of soil bacteria increases after conversion, communities become more similar across space (Rodrigues et al., 2013; Figuerola et al., 2015). The homogenization of microbial communities in response to human activities is driven by the loss of soil bacteria with restricted ranges (endemics) from the natural systems and results in a net loss of diversity (Rodrigues et al., 2013; Figuerola et al., 2015). As soil microbial diversity drives multiple ecosystem functions related to plant productivity (Delgado-Baquerizo et al., 2016a), we argue that microbial biodiversity loss (through homogenization of microbial community) should be taken into account when assessing the impact of land use change.

Relative Abundance of Major Bacterial Phyla in Agricultural vs. Natural Systems

In our analysis, despite the complex nature of soil microbial communities, we found general patterns characterizing microbial community responses to land use change at the continental scale which can provide strong framework for future experiments to generate empirical evidence. Across all regions, the relative abundance of phylum *Acidobacteria* was significantly greater in natural ecosystems as compared to agricultural systems (Figure 5). In contrast the relative abundance of *Verrucomicrobia* was higher in agro-ecosystems in continental, temperate and tropical regions. Interestingly both of these groups are classified as “oligotroph” (r-strategists, Fierer et al., 2007; Trivedi et al., 2013) based on lower growth rates and on a preference for growing on relatively recalcitrant forms of C. Although both *Acidobacteria* and *Verrucomicrobia* seems to be dominant groups in soil, their ecology remains poorly understood as the members of these group are difficult to culture and study in the laboratory (Bergmann et al., 2011; Fierer et al., 2013). The negative effect of agricultural systems on *Acidobacteria* may be also related to higher pH in agro-ecosystems compared to natural ecosystems.

The relative abundance of *Proteobacteria* was higher in natural soils as compared to agricultural soils in all regions apart from arid regions (Figure 5). Many members of *Proteobacteria* are classified as plant growth promoting bacteria that facilitates nutrient acquisition and provides protection against diseases (Lugtenberg and Kamilova, 2009). Lower relative abundance of *Proteobacteria* in agricultural soils can thus have important implications for plant productivity and soil health. Interestingly, we observed that the decrease in proportion of *Cyanobacteria* was accompanied by an increased proportion of *Chloroflexi* in agricultural systems of Arid, Continental, and Temperate regions. The metabolic flexibility of *Chloroflexi* (Strauss and Fuchs, 1993) can provide a competitive advantage against *Cyanobacteria* for limiting nutrients or physical space when they co-occur in the same environment especially in fluctuating environmental conditions in agricultural soils. Change in the ratio between *Cyanobacteria* and *Chloroflexi* has been implicated to be the result of physical disturbances that lead to the destruction of the microscale topography, decreased N availability and likely altered soil moisture retention and soil surface albedo (Kuske et al., 2012). Therefore, the relationship between these two related phyla in soil environments has to be investigated in details to develop early warning tools for soil degradation.

Microbial Indicators of Soil Fertility and Primary Productivity

The results of our meta-analysis provide useful information about the global distribution of several groups of numerically abundant bacterial phyla in agricultural vs. natural systems across contrasting climatic regions. It was demonstrated that certain bacterial phyla responded differentially to the conversion of natural to agro-ecosystems and the trend was consistent across all studied regions. For example, the

relative abundance of *Acidobacteria* was higher in natural systems while the abundance of *Chloroflexi* was higher in agricultural systems. In our analysis, the dataset is derived from the relative abundance of major bacteria groups using next generation sequencing; however, previous studies have shown a significant correlation between the relative abundance and absolute numbers of major bacterial groups using qPCR (Trivedi et al., 2015). Our findings highlight the potential of molecular tools to identify bacterial groups that may serve as potential indicators to assess the sustainability of agricultural soil management and to monitor trends in soil condition over time.

It can be argued that the selection of indicator species based solely on the frequency of occurrence does not permit conclusions about the process in which they are involved (Figueroa et al., 2012). However, as discussed above the relative abundance of groups showing consistent trends in abundance in natural and agricultural systems across all the regions can be inferred by their trophic life-strategies and related to soil physio-chemical parameters. Therefore, it can be validly postulated that the abundance of these groups reflects true habitat specialization underlying ecological selection based on soil management. The abundance of the suggested bacterial phyla is easily measured since well-established molecular and conventional culturing protocols for quantification are available (Fierer et al., 2012); they are sensitive to soil management actions and are integrative, i.e., provide adequate coverage across a relatively wide range of ecological variables, soil types, climate, crop sequence, etc. Herein we provide a regional scale framework for developing appropriate tests for simple monitoring of proposed candidate biological indicators that can be integrated into a minimum dataset, to facilitate measuring the impact of agriculture on soil health. This will allow the development of base-line values and ranges to incorporate microbial indicators in management decisions. However, significant background work including identifying context of monitoring (aridity vs. productivity), selection parameters for biological indicators (positive or negative) need to be tested and validated before an efficient indicator of primary productivity can be developed for monitoring purpose.

CONCLUSION

We provide a detailed characterization of how bacterial communities change following the conversion of natural to agricultural systems, and reveal community-scale trends that hold across tropical, temperate, continental, and arid biomes. We propose that measures of microbial abundance may serve as indicators of changing to soil health before actual decline in physico-chemical properties are detected. Although our meta-analysis is derived from comprehensive datasets on the effect of agriculture on soil properties and the relative abundance of microbial taxa, this global dataset does not mirror the current hot spots of land use changes. New efforts are needed to quantify the effect of land use changes in South East Asia and Africa, also taking to account the carbon-rich wetland forests and

degradation cascades within land-use classes. Nevertheless our meta-analysis provides clear signals on the predictable nature of the microbial community responses to land-use types which can be used to conceptualize future studies on understanding of human decision-making for soil health and biodiversity.

AUTHOR CONTRIBUTIONS

PT and MD-B designed this study and performed the meta-analysis in consultation with BS. Statistical analyses were done by PT and MD-B in consultation with BS and IA. PT wrote the article, with contributions from all co-authors.

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ACKNOWLEDGMENTS

We acknowledge the use of data from NEON, which are made available subject to the NEON Data Policy (www.neoninc.org). This research is supported by Grains Research and Development Corporation (project UWS00008) and Australian Research Council (project DP13010484).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fpls.2016.00990>

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Conflict of Interest Statement: 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.

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