Received: 29 January 2024 | Revised: 15 March 2024 | Accepted: 19 March 2024
DOI: 10.1111/gfs.12670

REVIEW ARTICLE

Grass and Forage Science — WILEY

The importance of multi-species grassland leys to enhance ecosystem services in crop rotations

C. S. Malisch[1] | J. A. Finn[2] | J. Eriksen[1] | R. Loges[3] | C. Brophy[4] | O. Huguenin-Elie[5]

[1]Department of Agroecology, Aarhus University, Tjele, Denmark
[2]Environment Research Centre, Teagasc, Wexford, Ireland
[3]Institute of Crop Science and Plant Breeding, Christian-Albrechts-University of Kiel, Kiel, Germany
[4]School of Computer Science and Statistics, Trinity College Dublin, Dublin 2, Ireland
[5]Forage production and grassland systems, Agroscope, Zurich, Switzerland

Correspondence
C. S. Malisch, Department of Agroecology, Aarhus University, Tjele, Denmark.
Email: cmalisch@agro.au.dk

Funding information
Horizon 2020 Framework Programme

Abstract

The ongoing simplification of agricultural production systems has resulted in several negative consequences, ranging from losses in soil organic carbon and biodiversity to a high dependency on external inputs to maintain high yields. We identify how grassland leys in crop rotations may help to mitigate these effects, by conserving soil organic carbon and enhancing nutrient efficiency. In particular, grasslands containing legumes enhance these benefits by providing nitrogen, and displacement of mineral N fertilizer. In crop rotations, these grasslands may transfer some of the acquired nitrogen to arable follow-on crops, thereby reducing the necessity for external inputs, while at the same time providing additional benefits, such as improvement of soil quality and reduction of weed pressure. However, there are still considerable knowledge gaps about how to optimize the community composition of grassland leys to best enhance the supply of these ecosystem services. Although the benefits of multi-species grasslands for the grassland crop have been shown repeatedly and across a large gradient of environments, further research is required to determine the benefits for follow-on crops, particularly across different environmental conditions. Here, we emphasize the importance of multi-site research, such as in the research network LegacyNet. Finally, we present management techniques that are optimized for both ecosystem services and agronomic performance in mechanically cut and grazed systems. For the latter, we consider how the inclusion of bioactive plant species can enhance animal health and lower methane emissions in grazing ruminants.

KEYWORDS
circularity, crop rotations, diversification, LegacyNet, nutrient cycling, sustainability

1 | INTRODUCTION

Agricultural production systems have continuously become more specialized, thereby enabling an intensified production, and today less than 10% of the agricultural areas of the United States and western,

An earlier version of this article was a keynote presentation at the 22th Symposium of the European Grassland Federation hosted by Lithuania in 2023 (www.europeangrassland.org/).

central and northern Europe are used for integrated crop livestock systems (Garrett et al., 2020). However, this led to an ongoing simplification of production systems at both temporal (i.e. monocropping) and spatial (monocultures and simplified landscapes) level. As a result, many regions developed high densities in either continuous cropping of arable crops, or livestock production with high-energy, low-fibre rations that often contain a large share of grain produced off-farm. Due to the arising spatial separation, manure often needs to be processed and transported to regions with lower animal density at substantial cost to avoid excessive nutrient loads to groundwater. The processing and enhanced storage duration of manure increases ammonia volatilisation, thereby reducing the nitrogen (N) efficiency of dairy farms, sometimes to as low as 20% (Löw et al., 2020). Simultaneously, N in the form of mineral fertilizer is applied on the utilized agricultural area; on average across the EU this amounts to 72 kg ha^{-1} yr^{-1} although there are large regional differences (de Vries et al., 2021). A more diversified approach based on integrated crop livestock systems would reduce these requirements for manure movement and mineral N fertilization. Thus, while increasing yields per unit area, the simplification of agricultural systems has resulted in severe negative consequences, ranging from losses in soil organic carbon to a high dependency on external inputs (Lemaire et al., 2015). For example, global cereal yields have increased by almost 4-fold between 1961 and 2016, during the same time, global mineral N fertilizer production has increased by almost a factor of 10, and amounted to 123,000,000 tonnes N annually by 2018 (Kopittke et al., 2019). This is problematic for various environmental reasons, for instance the production of each kg of N fertilizer produces greenhouse gas (GHG) emissions in the range of 1.3–4 kg CO_{2eq} kg^{-1} N for urea, and 3.5–10.3 kg CO_{2eq} kg^{-1} N for ammonium nitrate (Walling & Vaneeckhaute, 2020). The differences in emission factors hereby are largely attributable to the energy source during production and the state of production process, with Europe generally producing mineral fertilizers with lower footprints compared to China and the United States,

Similarly, the specialization of agriculture has resulted in substantial losses of soil organic carbon in the regions dedicated to crop production. Moreover, the increasing amount of concentrate feed in ruminant production systems is understood to have resulted in reduced grassland use throughout Europe (van den Pol-van Dasselaar et al., 2020), and thus have contributed to losses of soil organic carbon. This has direct implications for soil organic carbon stocks for three reasons: (a) the amount of mechanical disturbance is reduced substantially in grasslands, thereby allowing less soil organic carbon oxidation and mobilization, (b) grasslands have a dense rooting system, where more of the total biomass is stored belowground compared to annual crops, and even more so in species-rich grasslands, and (c) grasslands have a year-round soil cover with no bare ground being exposed to erosion. Thus, crop rotations with grassland leys are advocated to be a viable alternative that produces both arable crops and forage, while also maintaining carbon stocks. However, little is known about the general applicability of these findings across environmental conditions, ley community composition and management types. Consequently, our goal is to address the following research questions:

a. Can higher diversity in grassland leys increase ecosystem service performance and affect agronomic performance of the follow-on crop?
b. How can multisite experiments help better investigate the impact of management and environmental conditions on the effects of grassland leys?
c. How should grassland mixtures be designed and managed to balance ecosystem services and agronomic potential? and
d. What is the future outlook for diverse grassland leys?

2 | REASONS FOR INTRODUCING GRASSLAND LEYS

Historically, leys hugely contributed to improve agricultural production by replacing the grazed fallow of the medieval three-field system (Stebler, 1895). It is therefore obvious that expectations of leys include a high production of high-quality forage, as well as the maintenance of an adequate soil structure for cropping (Hoeffner et al., 2021). As an important element of the crop rotation, it is moreover expected that leys suppress crop weeds and soil-borne diseases (Martin et al., 2020). For instance, Dominschek et al. (2021) observed that weed biomass in maize (*Zea mays* L.) was more reduced after three years of ley than after a sunflower (*Helianthus annuus* L.)-maize-sunflower sequence because the relative abundance of fast-growing weeds was suppressed. Because ley cropping allows the selection of a mix of forage species and varieties with specific properties, it allows the design of forage mixtures that prioritize various functions, types of utilization and pedo-climatic conditions (Lüscher et al., 2019). For commercial grassland seed mixtures, the range of utility nevertheless needs to remain broad enough for economic efficiency. Lately, resilience to severe weather events, resource use efficiency and multifunctionality have received increased attention, as well as the potential role of plant diversity within the leys to target these multiple functions. Here, we review evidence of the effects of plant diversity on the main roles of leys.

3 | INCREASING DIVERSITY: IMPROVED PERFORMANCE OF MULTISPECIES GRASSLANDS COMPARED TO MONOCULTURES

3.1 | Forage quality for livestock

Growing grass and legume species in the same sward can provide an improved balance of forage in terms of its energy and protein contents compared with that of pure swards of either grasses or legumes (Dewhurst et al., 2009; Lüscher et al., 2014). Forage digestibility and protein content decrease with increasing plant maturity and the associated formation of structural tissues. Thus, for a given sward, forage digestibility and protein content usually decrease with increasing yield. In a study by Jing et al. (2017), indeed diverse mixtures with 10 or 12 species had higher yields than mixtures with lower diversity (3 species), especially in the third or fourth experimental year. At the same time, digestibility was generally slightly lower in the diverse mixtures, which was mostly a result of lower grass shares, and the legume proportion being dominated by lucerne, which had a lower digestibility. Similarly, White et al. (2004) identified higher species densities reduced in vitro organic matter digestibility (IVOMD) especially in autumn, independent of whether the swards were used in continuous grazing or a high intensity rotational grazing. The legume shares in even the most diverse mixtures generally ranged, however, between 2% and 27%, with the vast majority having legume shares of below 15% and non-leguminous dicots generally being well below 5%. Thus, established botanic composition is more important than sown diversity, as shown in a mob grazing experiment in which a three- and nine-species mixture did not differ in digestibility, but also not in botanic composition as only three species persisted in the diverse mixture (Deak et al., 2007). Additionally, the above studies all compared high diversity swards with low (generally 3 species) diversity plots. In a comparison of equi-proportional six-species mixtures and monocultures of perennial ryegrass, where both were fertilized with 150 kg N ha^{-1}, the diverse mixtures had a higher organic matter digestibility with 66.1% and 62.6% respectively (Khan et al., 2023). Similarly, in a mixture experiment using up to four-species mixtures, forage yield in grass-legume mixtures was higher without compromising forage digestibility and protein content (Sturludóttir et al., 2014; Suter et al., 2021). Furthermore, positive mixture effects on forage voluntary intake by livestock can compensate for reductions in digestibility (Baker et al., 2023; Roca-Fernández et al., 2016). These effects have for example been observed with a binary mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), compared to perennial ryegrass and chicory (*Cichorium intybus* L.) mixtures (Niderkorn et al., 2017; Niderkorn et al., 2019). Similarly, Soder et al. (2007) observed that adding clover to a grass monoculture increased DM intake, and that within these mixtures higher clover shares also resulted in higher intakes. Further increasing the mixture complexity from two, to three, six or nine species did not affect the ingestive grazing behaviour of dairy cows (grazing time, biting rate, and grazing jaw movements; Soder et al., 2006). In Loza et al. (2021), dairy cows grazing a complex eight-species mixture even increased milk yields compared to those cows grazing a simple binary mixture, which was likely a result of their higher herbage intake. Also Grace et al. (2019) showed that lambs grazing diverse pastures had higher liveweight gains than those grazing monocultures, and this was attributed to a combination of comparable or partially higher digestibility and lower parasite burdens. The latter were attributed to anthelmintic effects from several herbs and legumes, due to their natural concentration in plant specialized metabolites (PSM). Thus, in summary, Baumont and Niderkorn (2009) have already highlighted that while especially more in vivo data are required to resolve a current variability in the impact of multispecies mixtures on production, there are indeed indications that increased plant species diversity can benefit animal productivity, due to any of three modes of action – or a combination thereof: (i) faster particle breakdown of legumes results in higher passage rates and increased intake, ii) stimulation of microbial activity by enhanced N provision, and iii) secondary metabolites in legumes and herbs, which can affect the digestive process (more detail on that point can be found in the last section: "*Re-evaluating traditions and preparing them for the Future*").

3.2 | Benefits to yield across environmental conditions

The role of plant diversity in increasing yields has been reviewed recently (e.g. Jaramillo et al., 2021; Lüscher et al., 2022) and we briefly refer to it here. Climate change is already affecting both the mean and variance in climate conditions, with consequent increases in the incidence of severe weather events, including drought. Higher plant diversity within productive grassland communities is associated with higher drought resilience than less diverse mixtures or monocultures (Hofer et al., 2016; Komainda et al., 2020; Skinner, 2005). The strength of the relationship varies across studies and the degree of overyielding (i.e. the mixture has higher yields than the average monoculture yields of all species contained in that mixture, e.g Nyfeler et al., 2009) can be sufficiently strong for the more diverse mixture communities under drought to have similar or greater yields than the average of the monoculture yields in rainfed controls. Accordingly, Grange et al., 2021 have identified a six species mixture (consisting of two grasses, legumes and herbs each) fertilized with 150 kg N ha^{-1} and subjected to a two-months summer drought period using rainout shelters, to produce equal yields compared to the rainfed and irrigated best performing monoculture, and even compared to a ryegrass monoculture fertilized with 300 kg N ha^{-1} under rainfed and irrigated conditions. Similarly, Hofer et al., 2016, as well as Finn et al., 2018 identified equi-proportional four-species mixtures that were subjected to a nine-week summer drought using rainout shelters at three different sites to generally yield more biomass than the average of all monocultures under rainfed conditions. Under those summer drought conditions, plant diversity in mixtures also increased yield stability (Haughey et al., 2018; Macholdt et al., 2023). Finally, synergistic interspecific interactions can also occur in drought conditions, and mitigate drought effects on yield (see Lüscher et al., 2022).

3.3 | Nitrogen efficiency and losses

At the same level of moderate N fertilization, positive diversity effects on yield are usually produced by the interaction between grass and legumes (e.g. Nyfeler et al., 2009). With N availability being a decisive factor on drought tolerance of species, the ability of legumes to fix and transfer N also makes them an important tool to increase yield stability of grasslands under drought (Hofer et al., 2017). Furthermore, the presence of N_2-fixing legumes grown in association with grasses did not increase N leaching relative to that from pure grass swards when levels of N fertilization were adapted to crop requirements (Bracken et al., 2020). Moreover, legume-based multi-species leys may have a reducing effect on N_2O emissions (Peoples et al., 2019). Similarly, Cummins et al. (2021) found that N_2O emissions intensity from soil in six-species mixtures was 41% lower than that of a perennial ryegrass monoculture. Other trait contrasts that result in positive diversity impacts on yield are the combination of fast-establishing and persistent species or the combination of shallow- and deep-rooting species (Hofer et al., 2017; Husse et al., 2017); although these are not necessarily as strong as those associated with synergistic effects from grass-legume interactions (Finn et al., 2013). A further mechanism that could increase N efficiency of the ley is a more thorough capture of soil available N by vertical and temporal complementarity in N capture among the species of the community, induced by contrasting rooting depth and growth pattern (Husse et al., 2017). At the crop rotation level, the months following the destruction of the ley should also be included in N budgets, as N leaching is generally rather low during the period of ley cultivation (Valkama et al., 2016) but may be large following ley destruction (Eriksen et al., 2015; Hansen et al., 2019). Furthermore, residual N from ley cultivation can significantly reduce the need for fertilizer by the following crop (Fox et al., 2020; Grange et al., 2022).

3.4 | Can grassland mixtures show yield benefits within the restricted temporal duration of a ley?

Permanent grassland is commonly defined as grassland that is left unploughed for periods of at least five years. In multi-year experiments, the benefit of diversity generally increases over time. For example, van Ruijven and Berendse (2005) found no effect of diversity in the establishment year, but the effect was significant from year 2 onwards. Grassland leys, in contrast, are characterized by their short duration. While multi-species mixtures may have benefit for the yield of a follow-on crop, a key question is: can the known benefits of productive grassland mixtures over monocultures be evident on the yield of the ley within a period of twelve to eighteen months? Here, we look at field experiments that manipulated plant diversity in productive grasslands, and present evidence on the effect of mixture diversity within that time frame.

Overall, there are strong responses of mixture yields to diversity within an eighteen-month duration. In the AgroDiversity experiment that compared monocultures and mixtures of two grasses and two legumes across 31 different international locations, there were strong diversity effects and regular occurrence of transgressive overyielding (i.e. higher yields than the best performing species in the mixture grown as monoculture, e.g. Nyfeler et al., 2009) in the first full year of yield measurements after sowing (Kirwan et al., 2007). An associated experiment compared three N levels (Nyfeler et al., 2011), and found strong responses to plant diversity in the first year of measurements across all of them. An experiment across three sites that included four-species mixtures of a grass, two legumes and a herb also found strong yield responses to diversity in the first year of sampling (Hofer et al., 2016). A six-species experiment investigating communities assembled from two grasses, two legumes and two herbs also found strong responses to plant diversity in the first full year of yield measurements after sowing (Grange et al., 2021). In a five-site Norwegian study of five grasses and two legumes, species interactions led to overyielding (but not transgressive overyielding) in the first two years of the study on average across sites (Jørgensen et al., 2023). Using three-species mixtures of a grass, a legume and a herb, Cong et al. (2018) also showed strong yield responses to diversity in year 1, when the included herb was ribwort plantain (*Plantago lanceolata* L.). Similarly, Golińska et al. (2023) found evidence of overyielding (but not transgressive overyielding) in four species mixtures of grass, herb and legumes species across two contrasting soil types in the first year of their study. All of these studies were conducted under a harvesting regime and were not grazed.

With respect to the temporal duration of leys and diversity benefits, the temporal evolution of the botanical composition of the grasslands should also be considered. Indeed, more balanced mixtures tend to maximize the benefits of synergistic interspecific interactions and deliver the strongest yield responses. On the other hand, mixture compositions often tend to get less balanced over time. For instance, legume relative abundance often decreases from the first to the third year of ley cultivation in favour of grass (Brophy et al., 2017; Lüscher et al., 2014), with a corresponding decrease in ley performance. On the other hand, several benefits in a crop rotation, such as enhanced soil organic carbon, or a reduction in N leaching are generally considered to increase with increasing ley duration (Hu & Chabbi, 2022; Lemaire et al., 2015). Thus, there is a need to assess the optimal ley duration for maximal benefits from synergistic interspecific interactions.

3.5 | Flower resources for pollinators

Pollination is important for maintenance of wildflower communities and crop production as many crops are partially or fully dependent on insect pollination for seed or fruit set (Klein et al., 2006), but a historical decline is documented for wild pollinators in Europe (Biesmeijer et al., 2006). It is widely accepted that in most regions of the world, land management and pesticide use are important driving forces for these declines (Dicks et al., 2021). Therefore, multispecies grasslands are important in enabling a significant reduction in the use of herbicides (insecticide use is generally low in grasslands, (e.g. Lavery et al., 2021)), or even eliminate it if designed to support a higher diversity of pollinators by including plants with different pollinator profiles in flower mixtures to enhance flower-visiting insects (Cong et al., 2020). In a comparison of grassland leys with increasing levels of diversity compared to a conventional monoculture of perennial ryegrass as permanent grassland, a study by Beye et al. (2022) found that pollinator abundance increased drastically, with 541 wild bees of 10 species in the diverse grassland leys, compared to no wild bees in the permanent grassland due to the absence of flowers. Although pollinator abundance was not affected by the grassland ley diversity, the simple mixtures consisting of perennial ryegrass and white clover were only visited by common generalist species, whereas the multi-species grassland ley increased the abundance of rare long-tongued bumblebee to 10% in grazed areas, and even to 20% in ungrazed exclosures (Beye et al., 2022). Typically, legumes were visited mainly by large bees (honeybees and bumblebees) while some of the forbs attracted syrphids and other flies. Attracting pollinators with nectar delivering flowers must imperatively be combined with an adapted management, as mowing at an unsuitable time can amount to a fatal trap for the insects (Kenyeres & Varga, 2023). Spatially heterogeneous management can better support bees and hoverflies through delayed mowing and leaving uncut refuges in extensively managed grasslands (Meyer et al., 2017). An alternative to wildflower strips to achieve this is to systematically leave strips of uncut multispecies grassland at each mowing date, to be included in the following harvest (Cong et al., 2020). This may improve resource availability for pollinators considerably, with only marginal yield and quality loss.

4 | THE IMPACT OF A GRASSLAND LEY ON THE OUTCOMES OF A FOLLOW-ON CROP

4.1 | Nitrogen legacy effects of grassland leys

A substantial amount of research has investigated the abiotic and biotic consequences of conversion from grassland to arable crops (and vice versa) within crop rotations (Crème et al., 2018; Hoeffner et al., 2021; Martin et al., 2020). A variety of legacy effects are possible that include: the stocks and flows of specific nutrients (especially carbon and nitrogen); attributes of soil structure and hydrology; incidence of weeds, pests and pathogens and soil biodiversity. Yet, there has been much less research on how subsequent legacy effects are affected by plant diversity in a previous grassland ley.

Fox et al. (2020) used a systematically varying proportion of legumes across plant communities assembled from a grass, two legumes and a herb, and found that the yield of a follow-on monoculture of Italian ryegrass (*Lolium multiflorum* L.) (the legacy effect) was strongly related to the legume proportion in the preceding grassland community. Mixture communities with >20% legume proportion had a significantly higher legacy effect than that from grass monocultures. Communities with 50% legume proportion in the preceding grassland exerted the same legacy effect as a 100% legume proportion (legume monoculture), and the legacy effect was evident for at least 12 months. Similarly, in a six-species experiment by Grange et al. (2022) an experimental drought was imposed across all communities in the grassland phase, and compared with the rainfed control. All plots received 150 kg N ha^{-1} year^{-1} of N fertilizer (150 N) in the grassland phase, but were compared to a high N perennial ryegrass monoculture receiving 300 kg N ha^{-1} year^{-1} of N (300 N). They found a strong legacy effect that was related to the legume proportion in the grassland ley, and persisted across four successive yields over four months. The legacy effect was negatively affected by the drought, but to a modest degree in comparison to the effect of legume proportion. In this experiment, the legume proportion in the pre-crop was positively correlated with the benefits in the follow-on crop. In both studies, Fox et al. (2020) and Grange et al. (2022), was the effect of species diversity in the grassland stage on yield and N yield of the follow-on crop highly related, indicating that the highest yield benefits are likely a direct consequence of the highest N transfers to the follow-on crop. Thus at the crop rotation level, the highest N transfer to the follow-on crop was achieved with grassland mixtures containing a legume share around 50% (Fox et al., 2020) or even with legume monocultures (Grange et al., 2022). Consequently, the legume shares required for maximized N transfer to the follow-on crop are substantially higher than the legume shares required for maximal N yields within the grassland, where a legume share of 25%–33% has generally been sufficient to provide the maximum N yield across a large gradient of environments (Suter et al., 2015). Komainda et al. (2022) also observed that the yield of a follow-on crop of Italian ryegrass with a simple mixture as pre-crop was only 45% of the yield that was obtained when the pre-crop was a more complex lucerne-dominated multispecies grassland mixture.

4.2 | Additional benefits from diverse grasslands in a crop rotation

In the AgroDiversity experiment mentioned above, combining two grass and two legume species also strongly improved weed suppression during the period of ley cultivation, especially compared to the legume and grass monocultures (Connolly et al., 2018). For the function of weed suppression, the interaction between grass and legume species had the strongest positive effect, similar to that observed for yield, but the interaction between the fast-establishing and the persistent species played a major role as well. Thus, multi-species leys are more stable against weed invasion and might therefore help reduce the need for using herbicides. Additional benefits of grasslands in crop rotations have for example been illustrated by Colombi et al. (2017), who showed that in compacted soils, soil macropores from taproots are a tool to provide a path of least resistance, as well as oxygen, thereby enhancing yields of wheat (*Triticum aestivum* L.), maize and soybean (*Glycine max* (L.) Merr) as follow-on crops. However, Kautz et al. (2014) showed that even in non-compacted soils, taproots from chicory and lucerne (*Medicago sativa* L.) resulted in an increased density of biopores compared to tall fescue (*Festuca arundinacea* Schreb.) when grown for up to three years as a ley. In the same timeframe, anecic earthworms (*Lumbricus terrestris* L.) were unable to markedly increase the sub-soil's biopore density, indicating that in these ley durations, deep-rooting species with taproots may have a greater effect on the creation of biopores than earthworms (Kautz et al., 2014). In grass-clover swards with added manure, however, three years were sufficient to create biopores that aid the increased impact of the epi-anecic earthworm species *Aporrectodea longa* L. and *Lumbricus herculeus* L. (Krogh et al., 2021). These benefits have been shown before and are especially prominent in no-till systems, where the soil structure is not destroyed during the conversion of a follow-on crop (Alhameid et al., 2020). Yet, although little research has been conducted on the potential of multispecies leys directly to increase macropores via the inclusion of forages with deep taproots, similar studies on multispecies cover crops indicated their suitability to establish macropores in an even shorter timeframe, especially if these cover crops were grazed, thereby allocating more resources to form root biomass (Singh et al., 2021). We suggest there is need for further research on the potential of multispecies grassland leys with regard to their impact on macropore creation for follow-on crops.

5 | CONSIDERATIONS FOR THE SUCCESSFUL IMPLEMENTATION OF GRASSLAND MIXTURES IN CROP ROTATIONS

5.1 | Designing seed mixtures

A seed mixture may consist of a blend of varieties within a single species, as well as multiple species within a functional group, or combinations of species that vary in their functional group composition. The benefits of associating contrasting complementary traits (e.g. N_2-fixing root system of legumes with the efficient nutrient acquisition root systems of grasses) has been demonstrated in a number of studies (e.g. Finn et al., 2013; Husse et al., 2017; Nyfeler et al., 2011; Suter et al., 2021). While the highly contrasting traits between functional groups generally result in strong mixture effects, the extent of genetic diversity through the combining of complementary varieties within species may further improve the agronomic performances of the mixture (Meilhac et al., 2019; Prieto et al., 2015). While results from the Jena experiment indicate, however, that interspecific variability in traits is likely to more important than intraspecific trait plasticity (Roscher et al., 2018), it should be mentioned that in that study, intraspecific trait plasticity was derived from the performance of any species in mixture and monoculture, and from data from a single site experiment and one single sampling event. Other studies have found that especially when incorporating factors such as drought tolerance, intraspecific competition can outperform interspecific competition (Lüscher et al., 2022). This indicates that experiments with different cultivars are required that test the benefits from intraspecific variability across sites, environmental conditions and succession stages as well.

At the species level, experimental manipulations of plant diversity in productive grasslands from temperate regions suggest strong indications that (a) the identity of the plant species is crucial, and high-performing species should be targeted, and (b) more balanced mixtures tend to maximize the benefits of synergistic interspecific interactions and deliver the strongest yield responses in mixtures with three to six species from different functional groups of grasses, legumes and herbs (Lüscher et al., 2022). Especially strong mixing effects have been achieved with the targeted selection of two grasses and two legumes (Connolly et al., 2018; Finn et al., 2013). Higher species numbers often did not provide further benefits with regards to yield. Accordingly, Grange et al. (2021) identified that across two years, four-species equi-proportional mixtures consisting of only grasses and legumes provided comparable yields to six-species equi-proportional mixtures consisting of grasses, legumes and herbs. Similarly, in Hearn et al. (2024), three-species mixtures consisting of either grasses and legumes, or grasses, legumes and herbs, improved yields significantly in comparison to binary mixtures and monocultures, yet increasing the species number to five did not further increase yields. Haughey et al. (2018) tested the yield stability of up to four-species-mixtures including grasses, legumes and herbs across two sites and identified continuous benefits for yield stability when species diversity increased from one to four species. Thus, a large number of constituent species may not be required for maximized agronomic performances (Lüscher et al., 2022). This could also be the case for multifunctionality (Gamfeldt & Roger, 2017), as observed in the experiments of Dooley et al. (2015) where four-species mixtures (two grasses and two legumes) jointly enhanced biomass, weed suppression and N yield, and of Suter et al. (2021) in which four species (2 grasses and 2 legumes) were sufficient to induce concurrent diversity effects on yield, yield stability, weed suppression, symbiotic N_2 fixation and N efficiency, while maintaining forage quality.

This is in line with previous findings that the effect on biomass yield of adding a supplementary species has been shown to decrease with the number of species already included in the mixture (Weisser et al., 2017). This results from a combination of the yield reduction, which indicates that each added species increases the chance of including a species with lower yields than previously included species (Roscher et al., 2005), and the saturation curve that indicates that similarities in occupied ecological niches between species increase and reduce the yield gain from each additional species (Lüscher et al., 2022; Tilman et al., 1997). The saturation of ecological niches occurs with comparably few species in productive grasslands, compared to semi-natural grasslands (Freitag et al., 2023).

However, while mixtures containing up to four species appear to be sufficient to achieve the highest yields, the yield stability across sites and environmental conditions can be an issue. The variability in soil or climatic conditions, as well as the occurrence of successional stages over time, increase the number of ecological niches available. According to Lüscher et al. (2022), two strategies exist in order to enhance yield stability: the first strategy is to increase 'within-field diversity'. In this scenario, a larger number of species is included in the seed mixtures than will ultimately contribute yield in the field. Thus, always only a limited number of species might be present at any given time, yet the identity of these species can vary according to growth conditions and stages (Hooper et al., 2005). However, seeds from currently underutilized herbs and legumes can be considerably more expensive, with Schaub et al. (2021) identifying that diverse mixtures of 10 or 30 species cost on average 63% or 387% more, respectively, than a conventional agronomic monoculture. Consequently, considerations about the required species numbers are essential to balance the increased costs with benefits from the added species. Therefore, Lüscher et al. (2022) also highlights a second strategy, which is to increase 'among-field diversity'. In this strategy, simpler mixtures are designed for each climatic and soil conditions, which allows the use of less species, yet might be less flexible with regards to shifts in climatic conditions or climatic anomalies, as well as temporal successions. Therefore, with regards to the successful use across soil types and environments, further research is important that tests mixtures with more species in multi-site experiments across a gradient of environmental and soil conditions (see section below, 'Filling in the gaps…').

5.2 | Tailoring management for different ecosystem service priorities

Management is an important tool to steer the performance of swards and crop rotations between maximized yields and maximized ecosystem services. From an agronomic point of view, having grassland as the preceding crop in a rotation provides the highest benefits for the follow-on crop, combined with a ley duration that spans several years. Accordingly, in a comparison of the pre-crop effect in different crop rotations on spring triticale, the incorporation of a three-year old grass sward provided the highest grain DM yields, N yields and grain N contents. They were substantially higher than pre-cropping effects from the incorporation of an annual grass-clover sward, an arable legume (field bean) or even the annual fertilization of each crop in the rotation with either 100 or 200 kg N ha^{-1} in the form of cattle manure (Kayser et al., 2010). With regards to the reduction of environmental disservices, Nyfeler et al. (2024) identified nitrate leaching from an intact grassland to be equally low in grassland mixtures of four species (two grasses and two legumes) and grass monocultures, with nitrate concentrations in the soil solution (at 60 cm depth) being <0.2 mg NO_3^- -N L^{-1} across the 2nd and 3rd winter period of a 3-year grassland, independent of fertilization levels being 50 or 150 kg N ha^{-1} year^{-1}. In contrast, legume monocultures averaged concentrations of 8.1 and 11.5 mg NO_3^- -N L^{-1} over these periods, for 50 and 150 kg N ha^{-1}, respectively. Only at fertilization levels of 450 kg N ha^{-1} year^{-1} did the grass monoculture begin to outperform the mixtures with lower N concentrations in the leachate, and with legume monocultures still performing the worst. After ploughing, however, when looking at the soil mineral N (0–60 cm depth), the mixtures were generally lowest and were the only sward type to result in amounts below the critical threshold of 50 kg N ha^{-1} year^{-1}. Here, the four-species mixtures generally resulted in soil mineral N of 43 kg N ha^{-1}, compared to 77 kg N for the legume monoculture, in both cases independent of the fertilization being 50 and 150 kg N ha^{-1} year^{-1}. Contrary to that, the grass monoculture resulted in 50 and 63 kg for the fertilizer applications of 50 and 150 kg N ha^{-1} year^{-1} respectively. With regards to the fate of the N after ploughing, however, the choice of the follow on crop is also relevant, as the risk of leaching is lowest if the arable crop in the crop rotation was undersown with a catch crop to ensure an additional uptake of N in autumn (Biernat et al., 2020; Eriksen et al., 2015; Hansen et al., 2007). Accordingly, barley (*Hordeum vulgare* L.) with an undersown Italian ryegrass (the barley was harvested as whole crop silage to permit enough time for the Italian ryegrass to establish) was the only crop in a study by Eriksen et al. (2015) to result in low nitrate leaching of less than 10 kg N ha^{-1} year^{-1}, whereas maize as a follow-on crop resulted in high nitrate leaching amounts of more than 50 kg N ha^{-1} year^{-1} for five consecutive years.

Regarding the C inputs, grassland leys and permanent grassland can actually exhibit similar carbon inputs, but permanent grasslands retain the carbon longer in the soil; thus, only permanent grasslands result in substantially enhanced C stocks (Hu & Chabbi, 2022).

As a result, the modelling of changes in soil organic carbon (SOC) stocks, based on a 7 year experiment in Northern Germany and initial SOC stocks of around 50 Mg ha^{-1}, estimated annual losses across a 100 year time horizon to be on average 76 kg C ha^{-1} year^{-1} for the monocropping of silage maize, fertilized with 240 kg N ha^{-1} year^{-1} from cattle slurry, compared to gains in SOC of 413 kg C ha^{-1} year^{-1} in an equally fertilized permanent grassland. In that same experiment, a crop rotation consisting of two years of grassland, followed by maize and winter wheat resulted in slight annual gains of 15 kg C ha^{-1} (Loges et al., 2018). This accords with Jensen et al. (2022), who identified that introducing a 2-year-old grass-clover ley into a six-year arable crop rotation increased carbon stocks by 5 Mg ha^{-1}, before a new steady-state condition was achieved after 20 years. Subsequent conversion to a four-year grass-clover ley resulted in additional C stock increments of 4.2 Mg ha^{-1}, with no new steady state being achieved after 13 years. Similarly, Guillaume et al. (2022) have shown a clear benefit of leys for improved SOC, with a positive correlation between the proportion of leys in the crop rotation and SOC stocks (Figure 1). This is due to a combination of the negative impact of tillage on SOC stocks (Haddaway et al., 2017) and the fact that the root biomass only reaches its maximum in the second year (Weisser et al., 2017). After that peak, only grassland mixtures with a higher species richness were able to maintain high levels of root biomass, whereas grass monocultures had decreased their root biomass in year five by more than 50% compared to the first experimental year, and by approximately 75% compared to the maximum root biomass.

To balance soil C stocks and food production, Hu and Chabbi (2022) proposed the incorporation of grassland leys with high N application for three years embedded into a crop rotation. In their experiment, high N constituted on average 200–300 kg N ha^{-1} for the grassland stage, whereas the low N treatment received generally only 30 kg N ha^{-1}, and with the grassland being a mixture of three grasses only. Thus, with regards to the high N fertilization, this recommendation should be adjusted for mixtures and should also not be combined with grazing, as the combination of grazing and slurry application generally results in highest leaching rates in grasslands (Eriksen et al., 2015), whereas cutting generally produced the lowest leaching rates (Wachendorf et al., 2004). Accordingly, in a cut system, even N application rates of up to 300 kg N ha^{-1} did not increase N leaching in grass-clover swards of up to 4 years in age, even after the transition to an arable follow-on crop, while at the same time the continuous slurry application in the grass clover resulted in higher yield benefits effects for a follow-on crop if the grassland duration was longer than

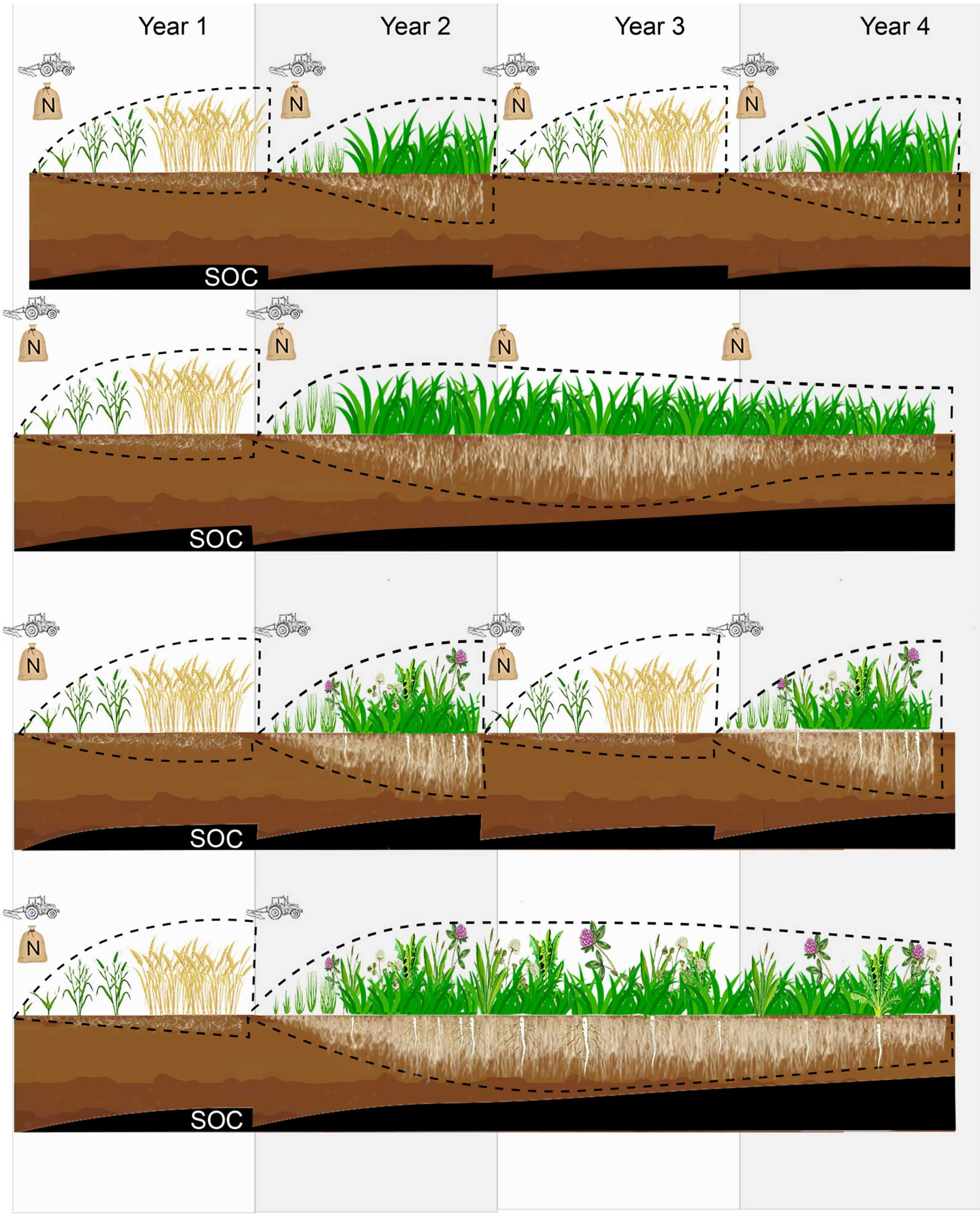

FIGURE 1 Conceptual representation of a crop rotation comprising a cereal crop followed by grassland leys of different duration (short ley: a, c; long ley: b, d) and species diversity (grass monocultures in the leys in panels a and b; multispecies grassland mixtures in panels c and d) on above- and belowground biomass production over time (dashed lines), as well as soil organic carbon (SOC) stock changes over time (black area at the bottom of each panel). Pictograms of tractors indicate ploughing events, while images of fertilizer bags of N indicate requirement of large amounts of external fertilizer applications. Overall, ley duration affects soil carbon stocks; legume-based mixtures reduce the requirement for nitrogen fertilizer inputs; mixtures maintain above- and below-ground biomass over time, and; grasslands have more root biomass than cereals.

2 years (Thers et al., 2024). However, N applications in excess of 200 kg N ha^{-1} did also not increase yields, but rather reduced legume shares and N fixation only (Thers et al., 2022). The ability to maintain fertilizer levels of around 200 kg N ha^{-1} (in the absence of livestock) without increasing leaching is especially important as reducing fertilizer amounts also reduces C sequestration, thereby resulting in a trade-off between C accumulation and N losses, as well as yields (Allard et al., 2007).

5.3 | Performance of diverse crop rotations is dependent on environmental and soil conditions

A recent meta-analysis from China has shown that the potential yield benefits of crop rotations are greatest when the following conditions are met: initial soil N concentrations are low; soil organic carbon stocks are intermediate, and; soil textures are coarse to intermediate (Zhao et al., 2022). This is to be expected, as potential yield gains are largest under conditions where nutrient status is most limiting, and can be resolved with nutrient management. However, environmental conditions also affect the soil microbial conditions and, with that, the turnover of residual biomass and the transfer of nutrients in a crop rotation. For example, in laboratory conditions, a study by Taghizadeh-Toosi et al. (2021) found that soil moisture levels of 60% water-filled pore space (WFPS) resulted in higher N mineralisation rates of red clover residues compared to 40% WFPS. Furthermore, a previous cropping history of diverse crop rotations instead of cereal monocropping has been shown to result in up to 80% higher recovery of N in follow-on crops, both under drought and controlled conditions (Bowles et al., 2022). As this is likely a result of changes in the soil microbial composition, soil–plant-microbial interactions will need to be analysed in detail to understand their impact on the N transfers. In addition to the direct impacts of cereal monocropping on the soil microbiome, the absence of diverse grassland swards also reduces macropores in the soil, which in turn impairs hydrological functions, and increased intensity of droughts and floods. The higher abundance of macropores is a direct consequence of especially diverse grasslands containing species with taproots, and of a higher abundance of earthworms in grassland leys compared to arable crops (Berdeni et al., 2021). These disturbances of hydrological functions further effect the soil microbiome and N transfers, as repeated drying-rewetting cycles might have particularly long-lasting impacts on C and N dynamics and increase N losses from the system via enhanced nitrifier activity (Fierer & Schimel, 2002).

5.4 | Filling in the gaps: New experimental approaches to account for multitude of factors affecting ecosystem services

The impact of environmental conditions, soil types and the resulting plant–soil-microbiome interactions require experimental approaches to account for these levels of complexity. If an experiment is repeated at multiple sites, the inference and generalisability of the results from the experiment can be greatly enhanced, compared to a single-site study. If multiple sites are selected at random across a country (or some alternative spatial scale) and a common experiment implemented at each site, the results can generalize to the scale of the country, whereas the results from a single-site study may be dependent on the conditions of the selected site. For example, in a grassland biodiversity experiment, the species diversity and other treatment outcomes may be affected by factors such as the soil type, previous land use history and climatic conditions at the site. Accordingly, in a previous 31-site experiment, where the performance of monocultures and mixtures of up to four species were tested, mixtures performed better than the average of the monocultures in 97% of the comparisons, and better than the best performing monoculture (i.e. transgressive overyielding) in approximately 60% of the sites, by up to almost 50% higher yields in the mixture compared to the best performing monoculture (Finn et al., 2013). Nevertheless, selecting a single-site experiment at random (reflecting single-site studies) could represent a wide range of outcomes within the range shown across sites, whereas the multisite experiment overcomes this limitation and allows the identification of generalizable trends, as well as ideally identifies reasons for the performance of mixtures across soil types and environmental conditions. However, the impact of these differences across sites requires a revised understanding of experimental, as well as analytical approaches.

LegacyNet is one example of a multi-site international experiment that attempts to utilize this multisite experiment to prioritize the potential generalization of the impact of grassland leys in crop rotations across environments and soil types (LegacyNet, 2024; O'Malley et al., 2023). For this, a common experiment was implemented at 32 sites across Asia, Europe, New Zealand and North America, and aims to understand how increased diversity in grassland leys can enhance not only the performance of the ley itself, but also that of a follow-on crop. Each LegacyNet site implements a grassland ley phase of at least 18 months, followed by a follow-on crop phase. In the grassland stage, systematically varying combinations of six forage species (two grasses, two legumes, two herbs), are established and dry matter and botanic composition and N concentration will be measured. The follow-on crop is then a monoculture of either a grass 'model' crop, or a cereal. Yield, N yield and other quality variables are recorded on each plot in this phase, as the project assumes that even the recording of a small number of responses can lead to deep knowledge increase, via the value from the distributed and coordinated data collection effort across multiple sites. This experimental work is ongoing and due to finish in 2024.

Thus, this approach enabled considerable variation of sites across geographical, environmental and soil conditions, and the results of this study will be powerful in testing the robustness of species diversity. Similar approaches will continue to be of utmost importance to overcome the limitations of single site experiments, due to the interconnectedness between soil, plants, management and climatic conditions.

5.5 | Re-evaluating traditions and preparing for the future

Bioactive herbs and legumes that contain tannins or other plant specialized metabolites (PSM) have been investigated as a promising route to be included in future grasslands. The PSM in these plants have been identified as having anthelmintic properties and to reduce methane emissions from ruminants and potentially enhance carbon stabilization rates in the soil (Adamczyk et al., 2017; Mueller-Harvey et al., 2018). The methane-reduction potential of tannins is considered especially promising, given that methane from livestock contributes substantially to anthropogenic GHG emissions; 17% of all food-system related emissions, and 5% of anthropogenic emissions (Arndt et al., 2022). In particular, tannins have the potential to mitigate these emissions by a combination of three modes of action: (1) they bind to fibres, thereby reducing their degradation and the formation of H_2 as substrate for methanogens, (2) they reduce protozoal activity, again affecting H_2 formation, and (3) they can directly inhibit methanogens (Baert et al., 2017). By exactly how much they can reduce the methane emissions is uncertain, due to a high variability in the findings, which originates from a large variability in both concentration and chemical structure of the tannins, both of which affect the bioactivity. In addition, methane emissions also vary substantially between animals, and there are additional cofounding factors such as the diet of the animals. Nonetheless, an overall pattern seems to emerge that indicates that proanthocyanidins (synonymous: condensed tannins) reduce methane emissions more compared to hydrolysable tannins, yet at the same time also exhibit higher reductions in dry matter digestibility as an unwanted side effect (Verma et al., 2021; Verma et al., 2022). Due to the variability and cofounding factors, as well as due to the differences within tannins and their working mode, general recommendations as to how high the tannin share should be for optimal bioactivity are no longer considered useful (Mueller-Harvey, 2006; Mueller-Harvey et al., 2019). An advantage is that tannin rich forages can immediately be incorporated into grasslands, as seed exists and no special technologies are required, thereby enabling a rapid potential for implementation, as well as the added benefits from incorporating herbs and legumes into grasslands, independent of their PSM concentrations. Furthermore, when tested with sheep, the palatability of several tannin rich species has been shown to be comparable to that of a ryegrass/clover mixture, when fed in dried form, or even partially exceed the ryegrass/clover palatability when fed as silage (Häring et al., 2008). Similarly, when grazing fresh birdsfoot trefoil as a tannin rich forage, neither experiments with dairy cows, nor with ewes resulted in a reduction of voluntary intake (Min et al., 2003). Consequently, their inclusion in sown grasslands could reduce the dependence on external inputs further, while generating healthy and nutritious diverse forages. However, to date, the agronomic potential of these bioactive species has often been low. Therefore, further efforts will be required to achieve a large share of continuously high bioactivity (Hamacher et al., 2021).

In the future, grasslands may also play a more important role in stockless plant production systems, not only for maintaining soil fertility and combat weed problems, but also for delivering bioenergy. There is an increasing interest in using grassland biomass for bioenergy production in Europe and North America (Prochnow et al., 2009; Wahid et al., 2018), which is further intensified with the current energy crisis. Biogas production can be enhanced through mixing of two or more substrates with complementary characteristics leading to improved efficiency of microorganisms involved in anaerobic digestion. It has been demonstrated that co-digestion of grass and forbs (chicory and plantain) synergistically enhanced methane yields (Cong et al., 2017). The synergistic effects were attributed to a more balanced nutrient composition (C/N ratio and micronutrients) in the grass-forb mixture promoting the utilization of multi-species grasslands for bioenergy production. Studies have shown that chicory and plantain generally contain higher mineral concentrations, such as P, Mg, K, S, Zn and B, than grass and legumes (Pirhofer-Walzl et al., 2011). It seems fair to conclude that biomass from especially unfertilized grass-clover-forb mixtures holds a large potential for greenhouse gas mitigation when used as biogas feedstock. Thus, all unfertilized grass-clover-forb mixtures met the EU sustainability criteria for renewable biofuels in the studies by Cong et al. (2017) and Carlsson et al. (2017), which provides an important incentive for developing bioenergy production systems based on such mixtures.

6 | CONCLUSION

Increasing species diversity from monocultures to mixtures of grasses, legumes and herbs can provide substantial benefits for grassland leys, by reducing external inputs and enhancing ecosystem services. The number of species that can be maintained in any system is, however, limited for intensive and semi-intensive production systems, and species-rich grassland seed mixtures currently come at a substantial premium price. Therefore, mixture designs need to balance the requirements for optimized yields, yield stability and ecosystem services, with the economic incentives to reduce the number of species in any mixture, all of this to provide good results across environmental and soil conditions. For this, the identification of both complementary traits, as well as redundancy effects between species and functional groups will be essential. As these effects are likely dependent on environmental and soil conditions, more multisite experiments will be required to understand these relations. This is even more so the case when expectations toward the grassland leys and the corresponding research questions include effects for a follow-on crop in a crop rotation. Here, grassland mixtures have exhibited similar benefits for a follow-on crop in a rotation as during the ley stage, with nutrient transfers to the follow-on crop, weed suppression and reduced losses in N leaching as some of the observed benefits. Yet, complex plant–soil-microbe interactions make it difficult to generalize and quantify these benefits. Additionally, the requirements in optimal species composition have been shown to vary between the grassland and follow-on crop stage, with generally higher legume shares being required for the highest yield benefits in the follow-on crop, than in the grassland ley itself. As future developments will, however, increase the importance of grasslands both in mixed and stockless plant production systems to close nutrient cycles and reduce external inputs, understanding the potential of grasslands in a crop rotation is a pressing matter. The improved understanding of the underlying processes, and opportunities and limits in using species diversity for a range of expected functions, will strengthen the role of multispecies grassland leys not only as excellent forage, but also to enhance the sustainability of agriculture as a whole.

ACKNOWLEDGEMENTS

This publication was supported by the European Union's Horizon 2021 doctoral network programme 'LegumeLegacy' under the Marie Skłodowska-Curie grant agreement No. 101072579. This invited review is an edited and updated version of the plenary paper prepared for the 22nd Symposium of the European Grassland Federation 'The Future Role of Ley-Farming in Cropping Systems'.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES

Adamczyk, B., Karonen, M., Adamczyk, S., Engström, M. T., Laakso, T., Saranpää, P., Kitunen, V., Smolander, A., & Simon, J. (2017). Tannins can slow-down but also speed-up soil enzymatic activity in boreal forest. *Soil Biology and Biochemistry*, *107*, 60–67. https://doi.org/10.1016/j.soilbio.2016.12.027

Alhameid, A., Singh, J., Sekaran, U., Ozlu, E., Kumar, S., & Singh, S. (2020). Crop rotational diversity impacts soil physical and hydrological properties under long-term no- and conventional-till soils. *Soil Research*, *58*(1), 84–94.

Allard, V., Soussana, J. F., Falcimagne, R., Berbigier, P., Bonnefond, J. M., Ceschia, E., D'hour, P., Hénault, C., Laville, P., Martin, C., & Pinares-Patino, C. (2007). The role of grazing management for the net biome productivity and greenhouse gas budget (CO_2, N_2O and CH_4) of semi-natural grassland. *Agriculture Ecosystems & Environment*, *121*(1–2), 47–58. https://doi.org/10.1016/j.agee.2006.12.004

Arndt, C., Hristov, A. N., Price, W. J., McClelland, S. C., Pelaez, A. M., Cueva, S. F., Oh, J., Dijkstra, J., Bannink, A., Bayat, A. R., Crompton, L. A., Eugène, M. A., Enahoro, D., Kebreab, E., Kreuzer, M., McGee, M., Martin, C., Newbold, C. J., Reynolds, C. K., … Yu, Z. (2022). Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5 °C target by 2030 but not 2050. *Proceedings of the National Academy of Sciences*, *119*(20), e2111294119. https://doi.org/10.1073/pnas.2111294119

Baert, N., Kim, J., Karonen, M., & Salminen, J.-P. (2017). Inter-population and inter-organ distribution of the main polyphenolic compounds of Epilobium angustifolium. *Phytochemistry*, *134*(Supplement C), 54–63. https://doi.org/10.1016/j.phytochem.2016.11.003

Baker, S., Lynch, M. B., Godwin, F., Boland, T. M., Kelly, A. K., Evans, A. C. O., Murphy, P. N. C., & Sheridan, H. (2023). Multispecies swards outperform perennial ryegrass under intensive beef grazing. *Agriculture, Ecosystems & Environment*, *345*, 108335. https://doi.org/10.1016/j.agee.2022.108335

Baumont, R., & Niderkorn, V. (2009). Associative effects between forages on feed intake and digestion in ruminants. *Animal*, *3*(7), 951–960. https://doi.org/10.1017/S1751731109004261

Berdeni, D., Turner, A., Grayson, R. P., Llanos, J., Holden, J., Firbank, L. G., Lappage, M. G., Hunt, S. P. F., Chapman, P. J., Hodson, M. E., Helgason, T., Watt, P. J., & Leake, J. R. (2021). Soil quality regeneration by grass-clover leys in arable rotations compared to permanent grassland: Effects on wheat yield and resilience to drought and flooding. *Soil and Tillage Research*, *212*, 105037. https://doi.org/10.1016/j.still.2021.105037

Beye, H., Taube, F., Lange, K., Hasler, M., Kluß, C., Loges, R., & Diekötter, T. (2022). Species-enriched grass-clover mixtures can promote bumblebee abundance compared with intensively managed conventional pastures. *Agronomy*, *12*(5), 1080. https://doi.org/10.3390/agronomy12051080

Biernat, L., Taube, F., Vogeler, I., Reinsch, T., Kluß, C., & Loges, R. (2020). Is organic agriculture in line with the EU-nitrate directive? On-farm nitrate leaching from organic and conventional arable crop rotations. *Agriculture, Ecosystems & Environment*, *298*, 106964. https://doi.org/10.1016/j.agee.2020.106964

Biesmeijer, J. C., Roberts, S. P. M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and The Netherlands. *Science*, *313*(5785), 351–354. https://doi.org/10.1126/science.1127863

Bowles, T. M., Jilling, A., Morán-Rivera, K., Schnecker, J., & Grandy, A. S. (2022). Crop rotational complexity affects plant-soil nitrogen cycling during water deficit. *Soil Biology and Biochemistry*, *166*, 108552. https://doi.org/10.1016/j.soilbio.2022.108552

Bracken, C. J., Lanigan, G. J., Richards, K. G., Müller, C., Tracy, S. R., Grant, J., Krol, D. J., Sheridan, H., Lynch, M. B., Grace, C., Fritch, R., & Murphy, P. N. C. (2020). Sward composition and soil moisture conditions affect nitrous oxide emissions and soil nitrogen dynamics following urea-nitrogen application. *Science of the Total Environment*, *722*, 137780. https://doi.org/10.1016/j.scitotenv.2020.137780

Brophy, C., Finn, J. A., Lüscher, A., Suter, M., Kirwan, L., Sebastià, M.-T., Helgadóttir, Á., Baadshaug, O. H., Bélanger, G., Black, A., Collins, R. P., Čop, J., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Ghesquiere, A., Golinska, B., … Connolly, J. (2017). Major shifts in species' relative abundance in grassland mixtures alongside positive effects of species diversity in yield: A continental-scale experiment. *Journal of Ecology*, *105*(5), 1210–1222. https://doi.org/10.1111/1365-2745.12754

Carlsson, G., Mårtensson, L.-M., Prade, T., Svensson, S.-E., & Jensen, E. S. (2017). Perennial species mixtures for multifunctional production of biomass on marginal land. *GCB Bioenergy*, *9*(1), 191–201. https://doi.org/10.1111/gcbb.12373

Colombi, T., Braun, S., Keller, T., & Walter, A. (2017). Artificial macropores attract crop roots and enhance plant productivity on compacted soils. *Science of the Total Environment*, *574*, 1283–1293. https://doi.org/10.1016/j.scitotenv.2016.07.194

Cong, W., Dupont, Y. L., Søegaard, K., & Eriksen, J. (2020). Optimizing yield and flower resources for pollinators in intensively managed multi-species grasslands. *Agriculture, Ecosystems & Environment*, *302*, 107062. https://doi.org/10.1016/j.agee.2020.107062

Cong, W.-F., Jing, J., Rasmussen, J., Søegaard, K., & Eriksen, J. (2017). Forbs enhance productivity of unfertilised grass-clover leys and support low-carbon bioenergy. *Scientific Reports*, *7*(1), 1422. https://doi.org/10.1038/s41598-017-01632-4

Cong, W.-F., Suter, M., Lüscher, A., & Eriksen, J. (2018). Species interactions between forbs and grass-clover contribute to yield gains and weed suppression in forage grassland mixtures. *Agriculture, Ecosystems & Environment*, *268*, 154–161. https://doi.org/10.1016/j.agee.2018.09.019

Connolly, J., Sebastià, M. T., Kirwan, L., Finn, J. A., Llurba, R., Suter, M., Collins, R. P., Porqueddu, C., Helgadóttir, Á., Baadshaug, O. H., Bélanger, G., Black, A., Brophy, C., Čop, J., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., … Lüscher, A. (2018). Weed suppression greatly increased by plant diversity in intensively managed grasslands: A continental-scale experiment. *The Journal of Applied Ecology*, *55*(2), 852–862. https://doi.org/10.1111/1365-2664.12991

Crème, A., Rumpel, C., Le Roux, X., Romian, A., Lan, T., & Chabbi, A. (2018). Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities. *Soil Biology and Biochemistry*, *122*, 203–210. https://doi.org/10.1016/j.soilbio.2018.04.018

Cummins, S., Finn, J. A., Richards, K. G., Lanigan, G. J., Grange, G., Brophy, C., Cardenas, L. M., Misselbrook, T. H., Reynolds, C. K., & Krol, D. J. (2021). Beneficial effects of multi-species mixtures on N_2O emissions from intensively managed grassland swards. *Science of the Total Environment*, *792*, 148163. https://doi.org/10.1016/j.scitotenv.2021.148163

de Vries, W., Schulte-Uebbing, L., Kros, H., Voogd, J. C., & Louwagie, G. (2021). Spatially explicit boundaries for agricultural nitrogen inputs in the European Union to meet air and water quality targets. *Science of the Total Environment*, *786*, 147283. https://doi.org/10.1016/j.scitotenv.2021.147283

Deak, A., Hall, M. H., Sanderson, M. A., & Archibald, D. D. (2007). Production and nutritive value of grazed simple and complex forage mixtures. *Agronomy Journal*, *99*(3), 814–821. https://doi.org/10.2134/agronj2006.0166

Dewhurst, R. J., Delaby, L., Moloney, A., Boland, T., & Lewis, E. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research*, *48*(2), 167–187.

Dicks, L. V., Breeze, T. D., Ngo, H. T., Senapathi, D., An, J., Aizen, M. A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L. A., Gemmill-Herren, B., Howlett, B. G., Imperatriz-Fonseca, V. L., Johnson, S. D., Kovács-Hostyánszki, A., Kwon, Y. J., Lattorff, H. M. G., Lungharwo, T., Seymour, C. L., … Potts, S. G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology & Evolution*, *5*(10), 1453–1461. https://doi.org/10.1038/s41559-021-01534-9

Dominschek, R., Barroso, A. A. M., Lang, C. R., de Moraes, A., Sulc, R. M., & Schuster, M. Z. (2021). Crop rotations with temporary grassland shifts weed patterns and allows herbicide-free management without crop yield loss. *Journal of Cleaner Production*, *306*, 127140. https://doi.org/10.1016/j.jclepro.2021.127140

Dooley, Á., Isbell, F., Kirwan, L., Connolly, J., Finn, J. A., & Brophy, C. (2015). Testing the effects of diversity on ecosystem multifunctionality using a multivariate model. *Ecology Letters*, *18*(11), 1242–1251. https://doi.org/10.1111/ele.12504

Eriksen, J., Askegaard, M., Rasmussen, J., & Søegaard, K. (2015). Nitrate leaching and residual effect in dairy crop rotations with grass–clover leys as influenced by sward age, grazing, cutting and fertilizer regimes. *Agriculture, Ecosystems & Environment*, *212*, 75–84. https://doi.org/10.1016/j.agee.2015.07.001

Fierer, N., & Schimel, J. P. (2002). Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, *34*(6), 777–787. https://doi.org/10.1016/S0038-0717(02)00007-X

Finn, J. A., Kirwan, L., Connolly, J., Sebastià, M. T., Helgadottir, A., Baadshaug, O. H., Bélanger, G., Black, A., Brophy, C., Collins, R. P., Čop, J., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Ghesquiere, A., Golinska, B., Golinski, P., … Lüscher, A. (2013). Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *Journal of Applied Ecology*, *50*(2), 365–375. https://doi.org/10.1111/1365-2664.12041

Finn, J. A., Suter, M., Haughey, E., Hofer, D., & Lüscher, A. (2018). Greater gains in annual yields from increased plant diversity than losses from experimental drought in two temperate grasslands. *Agriculture, Ecosystems & Environment*, *258*, 149–153. https://doi.org/10.1016/j.agee.2018.02.014

Fox, A., Suter, M., Widmer, F., & Luscher, A. (2020). Positive legacy effect of previous legume proportion in a ley on the performance of a following crop of Lolium multiflorum. *Plant and Soil*, *447*(1–2), 497–506. https://doi.org/10.1007/s11104-019-04403-4

Freitag, M., Hölzel, N., Neuenkamp, L., van der Plas, F., Manning, P., Abrahão, A., Bergmann, J., Boeddinghaus, R., Bolliger, R., Hamer, U., Kandeler, E., Kleinebecker, T., Knorr, K.-H., Marhan, S., Neyret, M., Prati, D., Le Provost, G., Saiz, H., van Kleunen, M., … Klaus, V. H. (2023). Increasing plant species richness by seeding has marginal effects on ecosystem functioning in agricultural grasslands. *Journal of Ecology*, *111*(9), 1968–1984. https://doi.org/10.1111/1365-2745.14154

Gamfeldt, L., & Roger, F. (2017). Revisiting the biodiversity–ecosystem multifunctionality relationship. *Nature Ecology & Evolution*, *1*(7), 0168. https://doi.org/10.1038/s41559-017-0168

Garrett, R. D., Ryschawy, J., Bell, L. W., Cortner, O., Ferreira, J., Garik, A. V. N., Gil, J. D. B., Klerkx, L., Moraine, M., Peterson, C. A., Reis, J. C. D., & Valentim, J. F. (2020). Drivers of decoupling and recoupling of crop and livestock systems at farm and territorial scales. *Ecology and Society*, *25*(1), 24. https://doi.org/10.5751/ES-11412-250124

Golińska, B., Vishwakarma, R., Brophy, C., & Goliński, P. (2023). Positive effects of plant diversity on dry matter yield while maintaining a high level of forage digestibility in intensively managed grasslands across two contrasting environments. *Grass and Forage Science*, *78*(4), 438–461. https://doi.org/10.1111/gfs.12644

Grace, C., Lynch, M. B., Sheridan, H., Lott, S., Fritch, R., & Boland, T. M. (2019). Grazing multispecies swards improves ewe and lamb performance. *Animal*, *13*(8), 1721–1729. https://doi.org/10.1017/S1751731118003245

Grange, G., Brophy, C., & Finn, J. A. (2022). Grassland legacy effects on yield of a follow-on crop in rotation strongly influenced by legume proportion and moderately by drought. *European Journal of Agronomy*, *138*, 126531. https://doi.org/10.1016/j.eja.2022.126531

Grange, G., Finn, J. A., & Brophy, C. (2021). Plant diversity enhanced yield and mitigated drought impacts in intensively managed grassland communities. *Journal of Applied Ecology*, *58*(9), 1864–1875. https://doi.org/10.1111/1365-2664.13894

Guillaume, T., Makowski, D., Libohova, Z., Elfouki, S., Fontana, M., Leifeld, J., Bragazza, L., & Sinaj, S. (2022). Carbon storage in agricultural topsoils and subsoils is promoted by including temporary grasslands into the crop rotation. *Geoderma*, *422*, 115937. https://doi.org/10.1016/j.geoderma.2022.115937

Haddaway, N. R., Hedlund, K., Jackson, L. E., Kätterer, T., Lugato, E., Thomsen, I. K., Jørgensen, H. B., & Isberg, P.-E. (2017). How does tillage intensity affect soil organic carbon? A systematic review. *Environmental Evidence*, *6*(1), 30. https://doi.org/10.1186/s13750-017-0108-9

Hamacher, M., Malisch, C. S., Reinsch, T., Taube, F., & Loges, R. (2021). Evaluation of yield formation and nutritive value of forage legumes and herbs with potential for diverse grasslands due to their concentration in plant specialized metabolites. *European Journal of Agronomy*, *128*, 126307. https://doi.org/10.1016/j.eja.2021.126307

Hansen, E. M., Eriksen, J., & Vinther, F. P. (2007). Catch crop strategy and nitrate leaching following grazed grass-clover. *Soil Use and Management*, *23*(4), 348–358. https://doi.org/10.1111/j.1475-2743.2007.00106.x

Hansen, S., Berland Frøseth, R., Stenberg, M., Stalenga, J., Olesen, J. E., Krauss, M., Radzikowski, P., Doltra, J., Nadeem, S., Torp, T., Pappa, V., & Watson, C. A. (2019). Reviews and syntheses: Review of causes and sources of N_2O emissions and NO_3 leaching from organic arable crop rotations. *Biogeosciences*, *16*(14), 2795–2819. https://doi.org/10.5194/bg-16-2795-2019

Haughey, E., Suter, M., Hofer, D., Hoekstra, N. J., McElwain, J. C., Lüscher, A., & Finn, J. A. (2018). Higher species richness enhances yield stability in intensively managed grasslands with experimental disturbance. *Scientific Reports*, *8*(1), 15047. https://doi.org/10.1038/s41598-018-33262-9

Hearn, C., Egan, M., Lynch, M. B., Dolan, K., Flynn, D., & O'Donovan, M. (2024). Can the inclusion of ribwort plantain or chicory increase the seasonal and annual dry matter production of intensive dairy grazing swards? *European Journal of Agronomy*, *152*, 127020. https://doi.org/10.1016/j.eja.2023.127020

Hoeffner, K., Beylich, A., Chabbi, A., Cluzeau, D., Dascalu, D., Graefe, U., Guzmán, G., Hallaire, V., Hanisch, J., Landa, B. B., Linsler, D., Menasseri, S., Öpik, M., Potthoff, M., Sandor, M., Scheu, S., Schmelz, R. M., Engell, I., Schrader, S., … Pérès, G. (2021). Legacy effects of temporary grassland in annual crop rotation on soil ecosystem services. *Science of the Total Environment*, *780*, 146140. https://doi.org/10.1016/j.scitotenv.2021.146140

Hofer, D., Suter, M., Buchmann, N., & Lüscher, A. (2017). Nitrogen status of functionally different forage species explains resistance to severe drought and post-drought overcompensation. *Agriculture, Ecosystems & Environment*, *236*, 312–322. https://doi.org/10.1016/j.agee.2016.11.022

Hofer, D., Suter, M., Haughey, E., Finn John, A., Hoekstra Nyncke, J., Buchmann, N., & Lüscher, A. (2016). Yield of temperate forage grassland species is either largely resistant or resilient to experimental summer drought. *Journal of Applied Ecology*, *53*(4), 1023–1034. https://doi.org/10.1111/1365-2664.12694

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Lodge, D. M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A. J., Vandermeer, J., & Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs*, *75*(1), 3–35. https://doi.org/10.1890/04-0922

Hu, T., & Chabbi, A. (2022). Grassland management and integration during crop rotation impact soil carbon changes and grass-crop production. *Agriculture, Ecosystems & Environment*, *324*, 107703. https://doi.org/10.1016/j.agee.2021.107703

Husse, S., Lüscher, A., Buchmann, N., Hoekstra, N. J., & Huguenin-Elie, O. (2017). Effects of mixing forage species contrasting in vertical and temporal nutrient capture on nutrient yields and fertilizer recovery in productive grasslands. *Plant and Soil*, *420*(1), 505–521. https://doi.org/10.1007/s11104-017-3372-0

Häring, D. A., Scharenberg, F., Heckendorf, F., Dohme, F., Lüscher, A., Maurer, V., Suter, D., & Hertzberg, H. (2008). Tanniferous forage plants: Agronomic performance, palatability and efficacy against parasitic nematodes in sheep. *Renewable Agriculture and Food Systems.*, *23*(1), 19–29. https://doi.org/10.1017/S1742170507002049

Jaramillo, D. M., Sheridan, H., Soder, K., & Dubeux, J. C. B. (2021). Enhancing the sustainability of temperate pasture systems through more diverse swards. *Agronomy*, *11*(10), 1912.

Jensen, J. L., Beucher, A. M., & Eriksen, J. (2022). Soil organic C and N stock changes in grass-clover leys: Effect of grassland proportion and organic fertilizer. *Geoderma*, *424*, 116022. https://doi.org/10.1016/j.geoderma.2022.116022

Jing, J., Søegaard, K., Cong, W.-F., & Eriksen, J. (2017). Species diversity effects on productivity, persistence and quality of multispecies swards in a four-year experiment. *PLoS One*, *12*(1), e0169208. https://doi.org/10.1371/journal.pone.0169208

Jørgensen, M., Bakken, A. K., Østrem, L., & Brophy, C. (2023). The effects of functional trait diversity on productivity of grass-legume swards across multiple sites and two levels of nitrogen fertiliser. *European Journal of Agronomy*, *151*, 126993. https://doi.org/10.1016/j.eja.2023.126993

Kautz, T., Lüsebrink, M., Pätzold, S., Vetterlein, D., Pude, R., Athmann, M., Küpper, P. M., Perkons, U., & Köpke, U. (2014). Contribution of anecic earthworms to biopore formation during cultivation of perennial ley crops. *Pedobiologia*, *57*(1), 47–52. https://doi.org/10.1016/j.pedobi.2013.09.008

Kayser, M., Müller, J., & Isselstein, J. (2010). Nitrogen management in organic farming: Comparison of crop rotation residual effects on yields, N leaching and soil conditions. *Nutrient Cycling in Agroecosystems*, *87*(1), 21–31. https://doi.org/10.1007/s10705-009-9309-0

Kenyeres, Z., & Varga, S. (2023). Effects of mowing on Isophya costata, natura 2000 species (orthoptera), by direct mortality and management history. *Journal of Insect Conservation*, *27*, 305–313. https://doi.org/10.1007/s10841-023-00456-0

Khan, A. S., Finn, J. A., Menezes, A. B. D., Kirwan, S. F., Waters, S. M., & Krol, D. J. (2023). Effects of multispecies and monoculture forages on nutrient digestibility and fermentation responses using an in vitro rumen simulation technique (RUSITEC). *Animal – Open Space*, *2*, 100052. https://doi.org/10.1016/j.anopes.2023.100052

Kirwan, L., Lüscher, A., Sebastia, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Golinski, P., Grieu, P., Gustavsson, A. M., Höglind, M., … Connolly, J. (2007). Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology*, *95*(3), 530–539.

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2006). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Komainda, M., Küchenmeister, F., Küchenmeister, K., Kayser, M., Wrage-Mönnig, N., & Isselstein, J. (2020). Drought tolerance is determined by species identity and functional group diversity rather than by species diversity within multi-species swards. *European Journal of Agronomy*, *119*, 126116. https://doi.org/10.1016/j.eja.2020.126116

Komainda, M., Muto, P., & Isselstein, J. (2022). Interaction of multispecies sward composition and harvesting management on herbage yield and quality from establishment phase to the subsequent crop. *Grass and Forage Science*, *77*(1), 89–99. https://doi.org/10.1111/gfs.12554

Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., & Lombi, E. (2019). Soil and the intensification of agriculture for global food security. *Environment International*, *132*, 105078. https://doi.org/10.1016/j.envint.2019.105078

Krogh, P. H., Lamandé, M., Holmstrup, M., & Eriksen, J. (2021). Earthworm burrow number and vertical distribution are affected by the crop sequence of a grass-clover rotation system. *European Journal of Soil Biology*, *103*, 103294. https://doi.org/10.1016/j.ejsobi.2021.103294

Lavery, M. K., Jess, S., Kirbas, J. M., Browne, A., & Matthews, D. (2021). *Pesticide usage survey report 308 – grassland and fodder crops in Northern Ireland 2021*. National Statistics. Retrieved from https://www.afbini.gov.uk/sites/afbini.gov.uk/files/publications/GRA21%20Final%20Report.pdf

LegacyNet. (2024). An international network of experiments. Retrieved from https://legacynet.scss.tcd.ie/

Lemaire, G., Gastal, F., Franzluebbers, A., & Chabbi, A. (2015). Grassland–cropping rotations: An avenue for agricultural diversification to reconcile high production with environmental quality. *Environmental Management*, *56*(5), 1065–1077. https://doi.org/10.1007/s00267-015-0561-6

Loges, R., Bunne, I., Reinsch, T., Malisch, C., Kluß, C., Herrmann, A., & Taube, F. (2018). Forage production in rotational systems generates similar yields compared to maize monocultures but improves soil carbon stocks. *European Journal of Agronomy*, *97*, 11–19. https://doi.org/10.1016/j.eja.2018.04.010

Loza, C., Reinsch, T., Loges, R., Taube, F., Gere, J. I., Kluß, C., Hasler, M., & Malisch, C. S. (2021). Methane emission and Milk production from Jersey cows grazing perennial ryegrass–White clover and multispecies forage mixtures. *Agriculture*, *11*(2), 175.

Löw, P., Karatay, Y. N., & Osterburg, B. (2020). Nitrogen use efficiency on dairy farms with different grazing systems in northwestern Germany. *Environmental Research Communications*, *2*(10), 105002. https://doi.org/10.1088/2515-7620/abc098

Lüscher, A., Barkaoui, K., Finn, J. A., Suter, D., Suter, M., & Volaire, F. (2022). Using plant diversity to reduce vulnerability and increase drought resilience of permanent and sown productive grasslands. *Grass and Forage Science*, *77*(4), 235–246. https://doi.org/10.1111/gfs.12578

Lüscher, A., Grieder, C., Huguenin-Elie, O., Klaus, V., Reidy, B., Schneider, M. K., Schubiger, F., Suter, D., Suter, M., Kölliker, R., & Kölliker, R. (2019). Grassland systems in Switzerland with a main focus on sown grasslands. *Grassland Science in Europe*, *24*, 3–16.

Lüscher, A., Mueller-Harvey, I., Soussana, J. F., Rees, R. M., & Peyraud, J. L. (2014). Potential of legume-based grassland–livestock systems in Europe: A review. *Grass and Forage Science*, *69*(2), 206–228. https://doi.org/10.1111/gfs.12124

Macholdt, J., Hadasch, S., Macdonald, A., Perryman, S., Piepho, H.-P., Scott, T., Styczen, M. E., & Storkey, J. (2023). Long-term trends in yield variance of temperate managed grassland. *Agronomy for Sustainable Development*, *43*(3), 37. https://doi.org/10.1007/s13593-023-00885-w

Martin, G., Durand, J.-L., Duru, M., Gastal, F., Julier, B., Litrico, I., Louarn, G., Médiène, S., Moreau, D., Valentin-Morison, M., Novak, S., Parnaudeau, V., Paschalidou, F., Vertès, F., Voisin, A. S., Cellier, P., & Jeuffroy, M.-H. (2020). Role of ley pastures in tomorrow's cropping systems. A review. *Agronomy for Sustainable Development*, *40*(3), 17. https://doi.org/10.1007/s13593-020-00620-9

Meilhac, J., Durand, J.-L., Beguier, V., & Litrico, I. (2019). Increasing the benefits of species diversity in multispecies temporary grasslands by increasing within-species diversity. *Annals of Botany*, *123*(5), 891–900. https://doi.org/10.1093/aob/mcy227

Meyer, S., Unternährer, D., Arlettaz, R., Humbert, J.-Y., & Menz, M. H. M. (2017). Promoting diverse communities of wild bees and hoverflies requires a landscape approach to managing meadows. *Agriculture, Ecosystems & Environment*, *239*, 376–384. https://doi.org/10.1016/j.agee.2017.01.037

Min, B. R., Barry, T. N., Attwood, G. T., & McNabb, W. C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Animal Feed Science and Technology*, *106*(1–4), 3–19. https://doi.org/10.1016/S0377-8401(03)00041-5

Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture*, *86*(13), 2010–2037.

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W. F., Salminen, J.-P., Skøt, L., Smith, L. M. J., Thamsborg, S. M., Totterdell, P., Wilkinson, I., Williams, A. R., Azuhnwi, B. N., Baert, N., Brinkhaus, A. G., … Waghorn, G. C. (2018). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science*, *59*, 861–885. https://doi.org/10.2135/cropsci2017.06.0369

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Lüscher, A., Niderkorn, V., Pellikaan, W. F., Salminen, J.-P., Skøt, L., Smith, L. M. J., Thamsborg, S. M., Totterdell, P., Wilkinson, I., Williams, A. R., Azuhnwi, B. N., Baert, N., Brinkhaus, A. G., … Waghorn, G. C. (2019). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science*, *59*(3), 861–885. https://doi.org/10.2135/cropsci2017.06.0369

Niderkorn, V., Martin, C., Bernard, M., Le Morvan, A., Rochette, Y., & Baumont, R. (2019). Effect of increasing the proportion of chicory in forage-based diets on intake and digestion by sheep. *Animal*, *13*(4), 718–726. https://doi.org/10.1017/S1751731118002185

Niderkorn, V., Martin, C., Le Morvan, A., Rochette, Y., Awad, M., & Baumont, R. (2017). Associative effects between fresh perennial ryegrass and white clover on dynamics of intake and digestion in sheep. *Grass and Forage Science*, *72*(4), 691–699. https://doi.org/10.1111/gfs.12270

Nyfeler, D., Huguenin-Elie, O., Frossard, E., & Lüscher, A. (2024). Effects of legumes and fertiliser on nitrogen balance and nitrate leaching from intact leys and after tilling for subsequent crop. *Agriculture, Ecosystems & Environment*, *360*, 108776. https://doi.org/10.1016/j.agee.2023.108776

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., & Lüscher, A. (2011). Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture Ecosystems & Environment*, *140*(1–2), 155–163. https://doi.org/10.1016/j.agee.2010.11.022

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, *46*(3), 683–691. https://doi.org/10.1111/j.1365-2664.2009.01653.x

O'Malley, J., Finn, J., Malisch, C., Adler, P., Bezemer, M., Black, A., Ergon, A., Eriksen, J., Filley, S., Fiorini, A., Golinski, P., Grange, G., Hakl, J., He, Y., Hockstra, N., Högy, P., Huguenin-Elie, O., Ibanez, M., Jiaxin, R., … Brophy, C. (2023). LegacyNet: Introducing an international multi-site experiment investigating potential benefits of increasing the species diversity of grassland leys within crop rotations. *Grassland Science in Europe*, *28*, 249–251.

Peoples, M. B., Hauggaard-Nielsen, H., Huguenin-Elie, O., Jensen, E. S., Justes, E., & Williams, M. (2019). Chapter 8 - the contributions of legumes to reducing the environmental risk of agricultural production. In G. Lemaire, P. C. D. F. Carvalho, S. Kronberg, & S. Recous (Eds.), *Agroecosystem diversity* (pp. 123–143). Academic Press.

Pirhofer-Walzl, K., Søegaard, K., Høgh-Jensen, H., Eriksen, J., Sanderson, M. A., Rasmussen, J., & Rasmussen, J. (2011). Forage herbs improve mineral composition of grassland herbage. *Grass and Forage Science*, *66*(3), 415–423. https://doi.org/10.1111/j.1365-2494.2011.00799.x

Prieto, I., Violle, C., Barre, P., Durand, J.-L., Ghesquiere, M., & Litrico, I. (2015). Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. *Nature Plants*, *1*(4), 15033. https://doi.org/10.1038/nplants.2015.33

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., & Hobbs, P. J. (2009). Bioenergy from permanent grassland – A review: 1. Biogas. *Bioresource Technology*, *100*(21), 4931–4944. https://doi.org/10.1016/j.biortech.2009.05.070

Roca-Fernández, A. I., Peyraud, J. L., Delaby, L., & Delagarde, R. (2016). Pasture intake and milk production of dairy cows rotationally grazing on multi-species swards. *Animal*, *10*(9), 1448–1456. https://doi.org/10.1017/S1751731116000331

Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N., Schulze, E.-D., & Schmid, B. (2018). Interspecific trait differences rather than intraspecific trait variation increase the extent and filling of community trait space with increasing plant diversity in experimental grasslands. *Perspectives in Plant Ecology, Evolution and Systematics*, *33*, 42–50. https://doi.org/10.1016/j.ppees.2018.05.001

Roscher, C., Temperton, V. M., Scherer-Lorenzen, M., Schmitz, M., Schumacher, J., Schmid, B., Buchmann, N., Weisser, W. W., & Schulze, E.-D. (2005). Overyielding in experimental grassland communities – Irrespective of species pool or spatial scale. *Ecology Letters*, *8*(4), 419–429. https://doi.org/10.1111/j.1461-0248.2005.00736.x

Schaub, S., Finger, R., Buchmann, N., Steiner, V., & Klaus, V. H. (2021). The costs of diversity: Higher prices for more diverse grassland seed mixtures. *Environmental Research Letters*, *16*, 094011. https://doi.org/10.1088/1748-9326/ac1a9c

Singh, N., Kumar, S., Udawatta, R. P., Anderson, S. H., de Jonge, L. W., & Katuwal, S. (2021). Grassland conversion to croplands impacted soil pore parameters measured via X-ray computed tomography. *Soil Science Society of America Journal*, *85*(1), 73–84. https://doi.org/10.1002/saj2.20163

Skinner, R. H. (2005). Emergence and survival of pasture species sown in monocultures or mixtures. *Agronomy Journal*, *97*(3), 799–805. https://doi.org/10.2134/agronj2004.0211

Soder, K. J., Rook, A. J., Sanderson, M. A., & Goslee, S. C. (2007). Interaction of plant species diversity on grazing behavior and performance of

livestock grazing temperate region pastures. *Crop Science*, *47*(1), 416–425. https://doi.org/10.2135/cropsci2006.01.0061
Soder, K. J., Sanderson, M. A., Stack, J. L., & Muller, L. D. (2006). Intake and performance of lactating cows grazing diverse forage mixtures. *Journal of Dairy Science*, *89*(6), 2158–2167. https://doi.org/10.3168/jds.S0022-0302(06)72286-X
Stebler, F. G. (1895). *Die Grassamen-Mischungen zur Erzielung des grössten Futterertrages von bester Qualität* (3 Auflage ed.). Druck und Verlag von K.J. Wyss.
Sturludóttir, E., Brophy, C., Bélanger, G., Gustavsson, A. M., Jørgensen, M., Lunnan, T., & Helgadóttir, Á. (2014). Benefits of mixing grasses and legumes for herbage yield and nutritive value in northern Europe and Canada. *Grass and Forage Science*, *69*(2), 229–240. https://doi.org/10.1111/gfs.12037
Suter, M., Connolly, J., Finn, J. A., Loges, R., Kirwan, L., Sebastià, M.-T., & Lüscher, A. (2015). Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Global Change Biology*, *21*(6), 2424–2438. https://doi.org/10.1111/gcb.12880
Suter, M., Huguenin-Elie, O., & Lüscher, A. (2021). Multispecies for multifunctions: Combining four complementary species enhances multifunctionality of sown grassland. *Scientific Reports*, *11*(1), 3835. https://doi.org/10.1038/s41598-021-82162-y
Taghizadeh-Toosi, A., Janz, B., Labouriau, R., Olesen, J. E., Butterbach-Bahl, K., & Petersen, S. O. (2021). Nitrous oxide emissions from red clover and winter wheat residues depend on interacting effects of distribution, soil N availability and moisture level. *Plant and Soil*, *466*(1), 121–138. https://doi.org/10.1007/s11104-021-05030-8
Thers, H., Jensen, J. L., Rasmussen, J., & Eriksen, J. (2022). Grass-clover response to cattle slurry N-rates: Yield, clover proportion, protein concentration and estimated N2-fixation. *Field Crops Research*, *287*, 108675. https://doi.org/10.1016/j.fcr.2022.108675
Thers, H., Jensen, J. L., Rasmussen, J., & Eriksen, J. (2024). Increasing cattle slurry application to grass-clover leys of different ages did not affect nitrate leaching but increased legacy effect in mixed organic crop rotations. *Field Crops Research*, *306*, 109233. https://doi.org/10.1016/j.fcr.2023.109233
Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., & Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science*, *277*(5330), 1300–1302.
Valkama, E., Rankinen, K., Virkajärvi, P., Salo, T., Kapuinen, P., & Turtola, E. (2016). Nitrogen fertilization of grass leys: Yield production and risk of N leaching. *Agriculture, Ecosystems & Environment*, *230*, 341–352. https://doi.org/10.1016/j.agee.2016.05.022
van den Pol-van Dasselaar, A., Hennessy, D., & Isselstein, J. (2020). Grazing of dairy cows in Europe—An in-depth analysis based on the perception of grassland experts. *Sustainability*, *12*(3), 1098.
van Ruijven, J., & Berendse, F. (2005). Diversity-productivity relationships: Initial effects, long-term patterns, and underlying mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(3), 695–700. https://doi.org/10.1073/pnas.0407524102
Verma, S., Taube, F., & Malisch, C. S. (2021). Examining the variables leading to apparent incongruity between Antimethanogenic potential of tannins and their observed effects in ruminants—A review. *Sustainability*, *13*(5), 2743.
Verma, S., Wolffram, S., Salminen, J.-P., Hasler, M., Susenbeth, A., Blank, R., Taube, F., Kluß, C., & Malisch, C. S. (2022). Linking metabolites in eight bioactive forage species to their in vitro methane reduction potential across several cultivars and harvests. *Scientific Reports*, *12*(1), 10454. https://doi.org/10.1038/s41598-022-14424-2
Wachendorf, M., Büchter, M., Trott, H., & Taube, F. (2004). Performance and environmental effects of forage production on sandy soils. II. Impact of defoliation system and nitrogen input on nitrate leaching losses. *Grass and Forage Science*, *59*(1), 56–68. https://doi.org/10.1111/j.1365-2494.2004.00401.x
Wahid, R., Feng, L., Cong, W.-F., Ward, A. J., Møller, H. B., & Eriksen, J. (2018). Anaerobic mono-digestion of lucerne, grass and forbs – Influence of species and cutting frequency. *Biomass and Bioenergy*, *109*, 199–208. https://doi.org/10.1016/j.biombioe.2017.12.029
Walling, E., & Vaneeckhaute, C. (2020). Greenhouse gas emissions from inorganic and organic fertilizer production and use: A review of emission factors and their variability. *Journal of Environmental Management*, *276*, 111211. https://doi.org/10.1016/j.jenvman.2020.111211
Weisser, W. W., Roscher, C., Meyer, S. T., Ebeling, A., Luo, G., Allan, E., Beßler, H., Barnard, R. L., Buchmann, N., Buscot, F., Engels, C., Fischer, C., Fischer, M., Gessler, A., Gleixner, G., Halle, S., Hildebrandt, A., Hillebrand, H., de Kroon, H., … Eisenhauer, N. (2017). Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: Patterns, mechanisms, and open questions. *Basic and Applied Ecology*, *23*, 1–73. https://doi.org/10.1016/j.baae.2017.06.002
White, T. A., Barker, D. J., & Moore, K. J. (2004). Vegetation diversity, growth, quality and decomposition in managed grasslands. *Agriculture, Ecosystems & Environment*, *101*(1), 73–84. https://doi.org/10.1016/S0167-8809(03)00169-5
Zhao, J., Chen, J., Beillouin, D., Lambers, H., Yang, Y., Smith, P., Zeng, Z., Olesen, J. E., & Zang, H. (2022). Global systematic review with meta-analysis reveals yield advantage of legume-based rotations and its drivers. *Nature Communications*, *13*(1), 4926. https://doi.org/10.1038/s41467-022-32464-0

How to cite this article: Malisch, C. S., Finn, J. A., Eriksen, J., Loges, R., Brophy, C., & Huguenin-Elie, O. (2024). The importance of multi-species grassland leys to enhance ecosystem services in crop rotations. *Grass and Forage Science*, *79*(2), 120–134. https://doi.org/10.1111/gfs.12670