

AGRONOMIC AND ENVIRONMENTAL BENEFITS OF REINTRODUCING HERB- AND LEGUME-RICH MULTISPECIES LEYS INTO ARABLE ROTATIONS: A REVIEW

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KEYWORDS

bioactive forages, integrated crop-livestock systems, nitrogen cycling, plant secondary metabolites, soil carbon, soil quality

HIGHLIGHTS

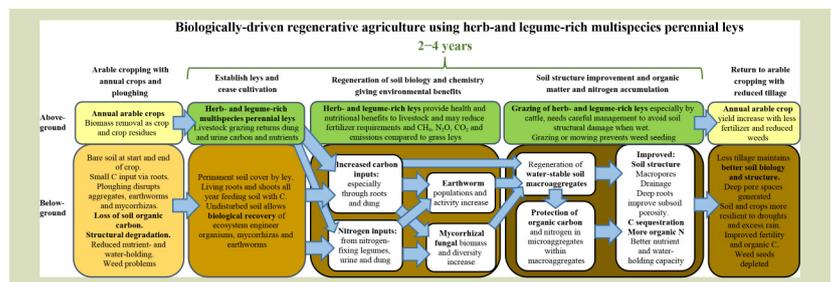
- Arable-ley rotations can alleviate soil degradation and erosion.
- Multispecies leys can improve livestock health and reduce greenhouse gas emissions.
- Ley botanical composition is crucial for determining benefits.
- Lack of livestock infrastructure in arable areas may prevent arable-ley uptake.
- Long-term (10–25 years) research is needed to facilitate evidence-based decisions.

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GRAPHICAL ABSTRACT



ABSTRACT

Agricultural intensification and the subsequent decline of mixed farming systems has led to an increase in continuous cropping with only a few fallow or break years, undermining global soil health. Arable-ley rotations incorporating temporary pastures (leys) lasting 1–4 years may alleviate soil degradation by building soil fertility and improving soil structure. However, the majority of previous research on arable-ley rotations has utilized either grass or grass-clover leys within ungrazed systems. Multispecies leys, containing a mix of grasses, legumes, and herbs, are rapidly gaining popularity due to their promotion in agri-environment schemes and potential to deliver greater ecosystem services than conventional grass or grass-clover leys. Livestock grazing in arable-ley rotations may increase the economic resilience of these systems, despite limited research of the effects of multispecies leys on ruminant health and greenhouse gas emissions. This review aims to evaluate previous research on multispecies leys, highlighting areas for future research and the potential benefits and disbenefits on soil quality and livestock productivity. The botanical composition of multispecies leys is crucial, as legumes, deep rooted perennial plants (e.g., *Onobrychis viciifolia* and *Cichorium intybus*) and herbs (e.g., *Plantago lanceolata*) can increase soil carbon, improve soil structure, reduce nitrogen fertilizer requirements, and promote the recovery of soil fauna (e.g., earthworms) in degraded arable soils

while delivering additional environmental benefits (e.g., biological nitrification inhibition and enteric methane reduction). Multispecies leys have the potential to deliver biologically driven regenerative agriculture, but more long-term research is needed to underpin evidence-based policy and farmer guidance.

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1 INTRODUCTION

With the global population expected to reach 10.9 billion by 2100, sustainable agricultural intensification is required to increase food production by 48.6% to meet the projected demand^[1,2]. However, current agricultural practices are unsustainable and have contributed to a global decline in soil quality, risking future crop and livestock production, especially with intensifying climate change^[3]. Reliance on agrochemicals and mineral fertilizers to increase productivity has led to increases in greenhouse gas (GHG) emissions^[4,5], pest and herbicide resistance^[6,7], loss of biodiversity^[8], and soil degradation^[9,10].

Soil degradation is widely recognized as a key threat to soils and the ecosystem services they provide^[11]. Due to their slow rate of formation, ca. 0.3–1.4 t·ha⁻¹·yr⁻¹ for Europe, soils are regarded as a non-renewable resource that need careful protection and management to preserve them for future use^[12]. Soils can provide multiple ecosystem services, such as carbon sequestration, food and fiber production, disease control, water quality management, and flood and climate regulation^[11,13]. Arable land management and cultivation practices, for example, deep tillage and removal of crop residue, can contribute to progressive soil degradation through damage to soil structure, loss of soil organic matter (SOM), increased compaction and an increased risk of erosion, especially in hilly regions. Poor soil structure also leads to more diffuse pollution; especially excessive nitrogen and increasingly phosphate leaching^[9]. Although it is difficult to accurately quantify the full economic impact of the loss of ecosystem services, soil degradation has a significant impact on the global economy. The 2006 Thematic Soil Strategy for the EU estimated the annual cost of soil degradation to be 38 billion EUR for the EU25 member states, with soil erosion and loss of soil organic matter costing 0.7–14.0 billion and 3.4–5.6 billion EUR·yr⁻¹, respectively^[14]. In a wider perspective, the global economic cost of land degradation has been estimated at 231 billion USD·yr⁻¹, equivalent to ~0.41% of the global GDP^[15].

Technological advancements in farm machinery, introduction of high-yielding crop varieties, increased consumer demand

and affluence, and government policies, subsidies and grants have improved farm efficiency and productivity per unit of labor but inadvertently accelerated the degradation of agricultural soils in many European nations^[16]. This has encouraged agricultural intensification and specialization, leading to the decline of mixed farming and the infrastructure to support this. Consequently, there has been an increase in monoculture farming and continuous cropping with no fallow or break years, which formerly involved rotations with grazed grass-clover leys, and subsequently decreasing the heterogeneity of the landscape and creating regional areas of soil degradation and environmental pollution^[17,18]. In the UK, for example, increases in specialization were initiated in 1947 by the introduction of the Agriculture Act following World War II to improve self-sufficiency^[17]. This resulted in a decline in mixed farming and a progressive geographical separation of land use with livestock-grazed grasslands becoming dominant in the wetter west of the country and arable farming becoming dominant in the drier east of the country^[16,19].

In many regions of the world, integrated crop-livestock systems, also referred to as mixed farming systems, have been reintroduced to promote more climate-resilient, sustainable and economically viable agricultural systems, compared to specialized and intensive systems^[20]. However, coupled crop-livestock farming systems maintain on-farm specialization but utilize neighboring farms to manage system inputs effectively (e.g., muck-for-straw deals), integrated crop-livestock systems utilize systems more efficiently and can produce higher economic returns than coupled crop-livestock farms^[21]. Integrated crop-livestock systems employ arable-ley rotations to alleviate soil degradation, improve soil quality for future use, build soil fertility via symbiotic nitrogen fixation in legumes, and increase resilience by diversifying the farm enterprise^[22,23]. Incorporating leys, temporary grasslands lasting up to 5 years, and integrating livestock into arable rotations can help to better manage arable weeds, pests, improve soil structure, enhance nitrogen fixation and recycle nutrients from livestock excreta back into the soil^[24]. However, the effect of integrated crop-livestock systems on soil quality and productivity can vary significantly depending on grazing management regime, soil type and ley species sown, for example, perennial ryegrass,

grass-legume, or a multispecies mix containing grasses, herbs and legumes^[25–27]. Ryegrass-based leys are often used due to their wide tolerance of different conditions, versatility of use for silage, hay, haylage and grazing, and high digestibility for livestock^[28], however, ryegrass monocultures provide limited ecosystem service benefits^[29,30]. By comparison, grass-legume leys have the benefit of reducing the need for mineral N fertilizers in the subsequent crop due to their N fixing abilities^[31]. It should be noted, however, that plowing in leys in preparation for the following crop can lead to increased N leaching into watercourses and thus indirect GHG emissions^[32]. Based on the study of natural ecosystems, however, it is clear that resilience and ecosystem delivery increases with plant diversity^[33]. Pasture management for increased plant species diversity, however, is not simply a case of mixing and planting as many forage species as possible. It is clear that the kinds and amounts of different forage species along with their arrangement within and among pastures at the farm scale are critical features that must be considered^[34].

Although research often focuses on the effect of multispecies leys (also known as herbal leys) on livestock health and productivity^[35], grass yield^[27] and GHG emissions^[36], there is currently limited information available on the combined effects of these leys on restoring soil quality in degraded arable soils over and above simple mixtures of two to three species. This review focuses on the potential benefits and implications of reintroducing multispecies leys and livestock grazing in arable rotations for (1) ecosystem services, (2) soil structure, (3) soil carbon and nitrogen cycling, (4) livestock GHG emissions, (5) livestock productivity, and (6) sustainable agriculture. Finally, we highlight areas that require further research.

2 REINTRODUCING LEYS INTO ARABLE ROTATIONS

2.1 Ecosystem services delivered by leys

Policy, land use and management regime heavily influence the ecosystem services that modern agriculture can provide. The decline in mixed farming, and subsequently the intensification of arable agriculture, has contributed to the generation of an unbalanced agroecosystem and the poor delivery of some ecosystem services. Such services are split into four core categories; provisioning (e.g., food and fiber production), regulating (e.g., climate and flood regulation), cultural (e.g., heritage and recreation), and supporting (e.g., nutrient cycling and biodiversity)^[37]. The drive to increase provisioning services to meet consumer demand often comes at the cost of

long-term regulating, supporting and cultural services^[38]. Disservices, for example, eutrophication from excessive nutrient use or species loss from agrochemical use, caused by intensive agriculture are not always experienced just at the local scale but can apply at a range of spatial scales impacting the wider ecosystem^[39]. These disservices are often described as trade-offs, where certain regulating or supporting services are reduced as a result of maintaining or increasing current food, fiber and bioenergy production^[39].

Although some agri-environment schemes (AES) encourage the use of amelioration measures to reduce disservices from agriculture, for example, promoting extensification or introduction of buffer strips, they have attracted criticism for increasing production pressure elsewhere to account for a reduction in provisioning services^[40,41]. In the UK, for example, the Higher Level Stewardship scheme in England, and the Tir Gofal and Glastir agri-environment schemes in Wales, promoted conversion of arable land to species-rich permanent pastures as an extensification measure^[42,43]. However, it is important to discriminate between the aims of restoring and establishing species-rich permanent pastures compared to the desired ecosystem services of establishing multispecies leys. Species-rich permanent pastures, such as described in the GS6 and GS7 scheme in England, aim to restore, maintain and protect important habitats such as lowland meadows and rush pastures^[44,45]. In contrast, schemes promoting the introduction of multispecies leys in arable rotations (e.g., GS4 scheme in England) aim to restore soil quality and provide new habitats (e.g., for pollinators and soil invertebrates)^[42]. However, as with other AES, farmer willingness and the voluntary nature of schemes is recognized as a key limitation to uptake^[46]. Further, assessment of the benefits of these schemes indicated that they provided little tangible improvement in key indicators such as biodiversity, carbon storage, greenhouse gas reduction, and water quality^[46,47]. Alternative strategies are therefore needed to promote current and future AES, particularly those that can be adopted at the landscape scale. In Ireland, an ongoing Results-Based Environment Agri Pilot Programme (REAP) is testing a results-based payment system to reward farmers for maintaining or improving their farm environment^[48]. Payments within this 2-year trial scheme are dependent on the results of an environmental scorecard, assessing ecological integrity (e.g., species richness), field margins (e.g., width) and the field boundary (e.g., hedgerow condition and density)^[48]. Farmers that establish multispecies leys within their grassland system can receive payments up to 275 EUR·ha⁻¹ if achieving the maximum scorecard result^[48]. However, currently REAP does not include tilled fields or multispecies leys sown within a crop rotation^[48]. This may be

an avenue that future AES explore to encourage uptake of multispecies leys to deliver multiple ecosystem services at a landscape level.

Although the establishment of species-rich pastures encourages improvements in biodiversity, it often fails to account for the persistent effects of previous intensive management on soil properties^[37,49]. This can limit the potential ecosystem services that the conversion of arable land to multispecies leys can offer. Incorporating leys and livestock into arable rotations offers the potential to increase provisioning services and ameliorate the disservices created by intensive arable agriculture. These potential services and disservices are illustrated in Fig. 1.

In Sweden, a system including a one-year grass ley in rotation did not encounter disservices between provisioning services and supporting or regulating soil services from the agroecosystem, but instead maximized the delivery of soil-focused ecosystem services^[50]. Under the zero N fertilizer regime, the grass ley system produced a greater yield than the

conventional continuous commodity crop system, which produced the least ecosystem services delivery overall^[50]. If a multispecies ley is introduced into cropping systems, it may provide a greater abundance of ecosystem services than a basic grass ley through increased resilience and complementarity of species^[41,51,52]. This was demonstrated in a 3-year, 31-site study across Europe where a four-species mixture consisting of two grasses and two legumes consistently outperformed the respective monoculture comparison of each plant species^[53]. This was attributed to the synergistic interactions between the plant types delivering transgressive overyielding and a greater resistance to weed invasion in the multispecies mixture than in the grass monoculture^[53]. During the 3-year study, only 4% of the total yield was weed biomass in the multispecies ley whereas the weed biomass of the monoculture mixtures increased from 15% to 32% over 3 years^[53]. The beneficial yield effects were also highlighted in Switzerland, where a temporary 3-year four-species mixture consisting of two grasses and two legumes receiving 50 kg·ha⁻¹ N delivered multifunctional ecosystem services at the same level as grass or legume

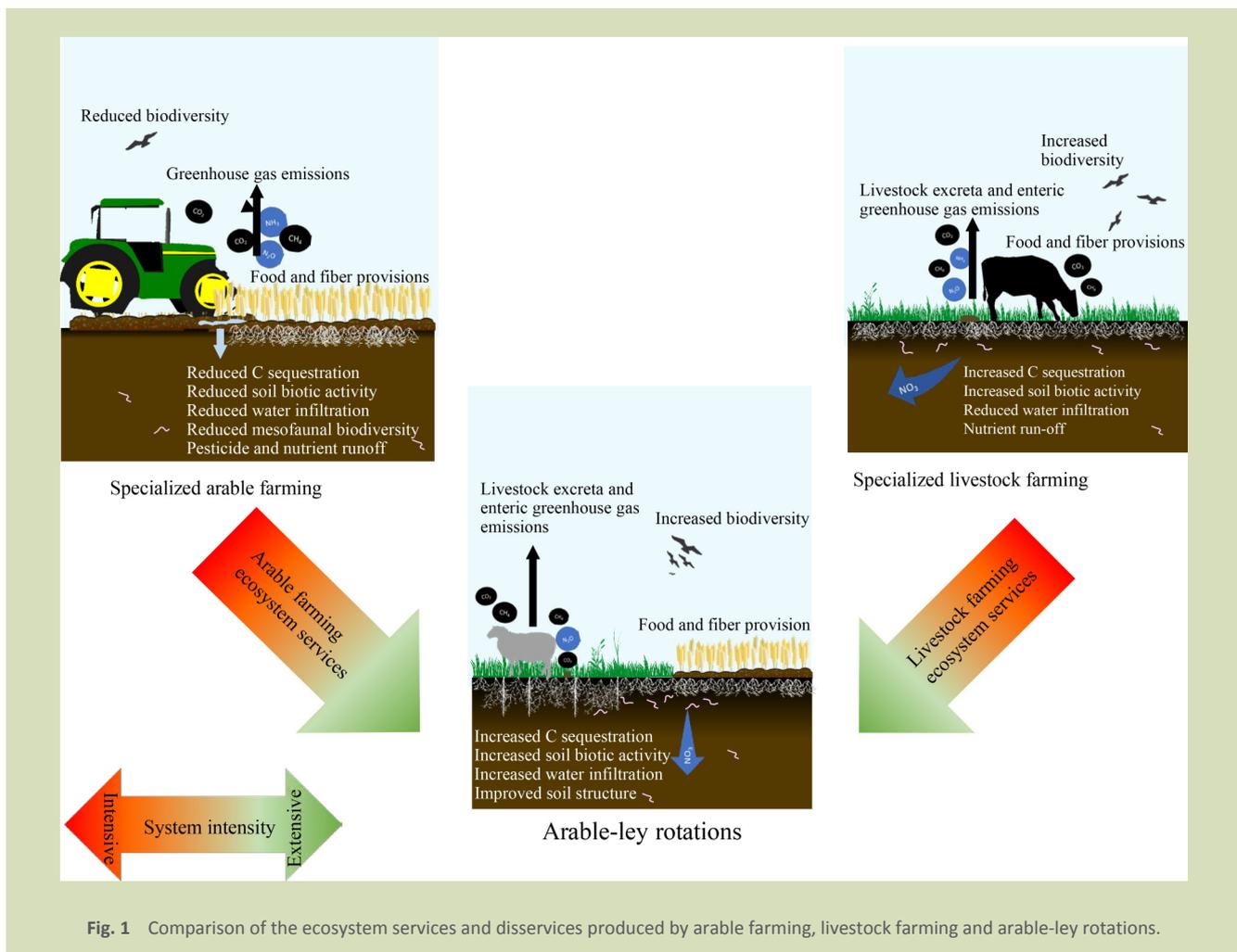


Fig. 1 Comparison of the ecosystem services and disservices produced by arable farming, livestock farming and arable-ley rotations.

monocultures receiving 450 kg·ha⁻¹ N^[54]. The greater delivery of ecosystem services such as N cycling, forage quality and production in the four-species mixture at a low N rate was mainly attributed to the symbiotic nitrogen fixation in legumes^[54]. Similar findings were reported in Ireland under rainfed conditions, where the annual yield of a 2-year six-species mixture receiving 150 kg·ha⁻¹ N outperformed the 2-year perennial ryegrass (*Lolium perenne*) monoculture receiving 300 kg·ha⁻¹ N by 1.3 t·ha⁻¹^[55].

In addition to the inclusion of nitrogen fixation from legumes in multispecies leys, attributes such as deep rooting of plant species including cocksfoot (*Dactylis glomerata*), *Festulolium*, chicory (*Cichorium intybus*), lucerne (*Medicago sativa*), sainfoin (*Onobrychis*), and sweet clover (*Melilotus officinalis*) can increase regulating and supporting services such as C storage, nitrogen fixation, soil structure and biodiversity^[56–59]. The deep rooting capabilities of these species enable access to water in deeper soil horizons to increase herbage production during dry periods compared to ryegrass leys, thus improving provisioning services^[50,60]. The benefits of species diversity under drought conditions were shown in Ireland by Grange et al.^[55], where a 2-year six-species mixture receiving 150 kg·ha⁻¹ N under drought conditions produced a similar yield (10.7 t·ha⁻¹) to a rainfed 2-year perennial ryegrass monoculture receiving 300 kg·ha⁻¹ N (10.5 t·ha⁻¹). The deep rooting capabilities of multispecies leys may also recover both macro- and micronutrients from depth which would have otherwise been lost. Although leys can be used for silage, hay or haylage production, grazing livestock on multispecies leys may increase farm productivity to address the economic gap that removing land from cultivation can cause. The supporting and regulating services that grazed multispecies leys in arable rotations can provide is explored in detail in the following sections. Although there has been extensive research into the delivery of ecosystem services in species-rich permanent grasslands, there is a lack of research into the ecosystem services delivered by temporary multispecies leys introduced to degraded arable land. Further research is needed to evaluate the potential benefits and disservices delivered by multispecies leys, with an emphasis on how these services can be maintained in the following crop.

3 IMPACT OF REINTRODUCING LEYS ON SOIL QUALITY

3.1 Soil quality under cropping systems

Application of agrochemicals for crop protection, use of

mineral fertilizers, tillage regime, high temperature and increased rainfall intensity are all factors that contribute to the loss of soil structure and soil fertility^[10,61,62]. Tillage is frequently used in arable agriculture to provide an effective seedbed, aid the decomposition of plant residues, and reduce crop pests, pathogens and weeds^[63]. However, continuous cultivation and intensive tillage practices such as moldboard plowing and harrowing frequently deplete SOC through enhanced oxidation, and also damage the soil crumb structure; reducing the macroporosity and contributing to greater compaction and erosion, which drive further soil degradation^[9–11]. The increased use of heavy farm machinery and the decline of mixed farming systems has increased reliance on mineral fertilizer inputs to maximize yield. The intensification of growing crop species that deliver only small amounts of organic matter that is stabilized in the soil has contributed to long-term depletion of SOM from arable soils, undermining soil stability that cannot be alleviated by normal tillage alone^[64]. Fine-textured arable soils with reduced SOM content are vulnerable to structural collapse when wet, and especially under compaction and prone to losses from water and wind erosion^[10]. Soils with poor aggregate stability suffer from increased susceptibility to water erosion that can lead to on-site and off-site environmental and economic impacts, such as reduced water holding capacity, loss of valuable nutrients such as N, P and K, reduced water quality, eutrophication, increased flood risk, and erosion leading to the siltation of watercourses and estuaries^[62]. Under current practices, it is estimated 112 Mha (12%) of European land area is under threat from water erosion, with a further 42 Mha affected by wind erosion^[14].

Maintaining a healthy soil structure is crucial in arable agriculture, as soil structure determines seedling establishment and root development, and thus nutrient use efficiency and yield^[62]. Tillage disrupts key biological processes responsible for soil structure formation and crop productivity. Arbuscular mycorrhizal fungal (AMF) hyphae, polysaccharides produced by microbial communities and mucilage excreted in earthworm casts act as an adhesive between soil particles and humus, forming micro- and macroaggregates and increasing soil stability and macropores that control infiltration rates^[65,66]. However, tillage can alter the composition and distribution of microbial communities in the soil profile, reduce earthworm populations and disrupt AMF hyphal networks, reducing their symbiotic ability to increase crop P uptake^[63,67–69]. The effects of tillage on earthworms is seen immediately after compaction events, where earthworm populations experience a 70% decline in total biomass due to animal death from crushing and lateral escape of the remaining

population^[69]. Effects of compaction on soil porosity can be seen for up to 2 years after the initial compaction event^[69]. This has consequences for the restoration of soil structure and porosity of arable soils, as earthworm burrows aid the mechanical working of the soil and create interconnected macro- transmission pores and channels that allow plant roots rapid access to nutrients at depth and influence water and air infiltration^[70,71]. Additionally, reduced soil porosity and inadequate drainage from compaction can create anaerobic conditions ideal for denitrifying bacteria, also favoring increased nitrous oxide (N₂O) production^[72].

Although some farmers attempt to ameliorate compaction and remove the plow pan through subsoiling, also referred to as deep tillage or subsoil ripping, this is fuel and labor intensive and creates a new soil structure inferior to that of uncompacted soil under grassland or reduced tillage management^[73]. Numerous studies and intergovernmental bodies have recognized the damaging impact excessive tillage has for soil degradation and encourage the adoption of alternative tillage methods, for example, minimum tillage (min-till) and no-tillage (no-till), to alleviate environmental issues^[74]. However, adoption of no-till methods remain slow in some areas due to concerns about soil compaction, reduction in pathogen inoculum, pest control (e.g., slugs), and perceived losses of crop productivity from herbicide resistant arable weeds, for example, black-grass (*Alopecurus myosuroides*), which would be buried deeper in the soil under an intensive tillage system reducing germination^[75,76]. Repeated herbicide and pesticide applications to control arable weeds and pests in no-till systems have contributed to a greater increase in herbicide resistant weeds, and herbicide and pesticide runoff into watercourses than multi-pass tillage systems^[77,78]. In the UK, common herbicide resistant weeds such as black-grass, wild oats (*Avena* spp.), Italian ryegrass (*Lolium multiflorum*), common poppy (*Papaver rhoeas*), common chickweed (*Stellaria media*), scentless mayweed (*Tripleurospermum inodorum*) and sterile brome (*Bromus sterilis*) threaten crop yield and farm productivity in arable systems^[78,79]. Development of herbicide resistant weeds, and recently the discovery of glyphosate-resistant Italian ryegrass^[80] and sterile brome^[79], has resulted in some no-till farmers returning to more intensive tillage^[81]. This has increased pressure to develop alternative pest management regimes for no-till systems. Incorporating leys and livestock, such as sheep and goats, into no-till and min-till systems can provide biocontrol for arable weeds, preventing seed set and thereby depleting seed banks over consecutive years, and pests without sacrificing the ecosystem services and improved soil structure of no-till^[82].

3.2 Improving soil quality using arable-ley rotations

Integrated crop-livestock systems have long utilized cover-crops, leys and livestock in arable rotations to ameliorate soil degradation without excessive chemical and mechanical inputs^[21]. Due to their ease of establishment and diversity of use, grass or grass-clover leys are used to improve arable soil structure, increase soil fertility, improve yield, and disrupt pest and pathogen life cycles^[83–85]. Most commonly, these leys are used for conservation, grazing or forage (e.g., hay or silage) production and are plowed into the topsoil after 1–4 years of use, losing some of the newly developed soil structure and accumulated SOC, and leaving soil and soil nutrients vulnerable to losses^[86]. Despite requiring increased herbicide inputs to remove competition from unwanted plant species, no-till management can help to preserve the improved soil structure and biological activity post-ley^[87].

Improvement of arable soil quality under ley is dependent on several key factors: ley duration, botanical composition, soil type, grazing density of livestock and agronomic management^[86]. It can take between 5 and 10 years for coarse sandy soils under an ungrazed grass ley to return to permanent pasture conditions and to up to 50 years for clay soils^[88]. Perennial ryegrass leys lack deep rooting capabilities, limiting their potential to bioturbate the soil and remove subsoil compaction. Perennial legumes and herbs, for example, chicory and lucerne, with deep primary roots (i.e., taproots) in the ley can influence soil microbial community composition, nutrient cycling, and increase soil porosity and infiltration through the generation of large pores (> 2 mm diameter) in the subsoil^[25,89,90]. Perennial plant taproots generate large continuous pores from the topsoil to the subsoil through root compression of soil particles and mucilage excretion from the root tip. Once decayed, this produces large pores, encouraging earthworm activity and root growth for the following crop and offering opportunities for subsoil C deposition and storage^[89,91,92]. However, it should be noted that short-term leys (< 3 years) may be insufficient to realize the synergies between deep rooted crops and deep burrowing earthworms^[89].

Inclusion of legumes in the ley composition can have persistent effects on soil, often observed to affect the following crop^[84]. Legumes can further encourage additional symbiotic relationships between N fixing bacteria in their root nodules and AMF, which enhances P sequestration in return for nitrogen fixation and plant assimilated C^[93,94]. This can increase the abundance of AMF hyphae and improve aggregate stability of arable soils under ley, encouraging an improvement in soil structure^[95]. Unlike arable soils where the AMF network

is regularly disrupted by frequent tillage, leys allow AMF to establish a new permanent network that can be preserved by no-till management during the establishment of the following crop^[87,93]. In Argentina, arable soils under a temporary grass-clover ley experienced a rapid restoration of soil properties (i.e., SOC and microbial biomass N) to original values within 3–4 years^[96]. Similarly, in New Zealand, microaggregates (< 0.25 mm) in arable soil under a temporary grass or grass-clover ley became highly water-stable macroaggregates (> 1 mm) after 5 years, attributed to the enmeshing of soil particles by grass roots and AMF hyphae^[95]. Introduction of leys into arable rotations allows the development of a denser root system that encourages increases in microbial biomass, earthworm and mesofauna activity, and subsequently the production of binding agents (e.g., mucilage and exopolysaccharides) which enable soil aggregate stability^[82].

Increases in earthworm populations in soils under leys can accelerate the restoration of degraded arable soils^[97]. Recovery of earthworm populations is relatively quick and increases with the duration of the ley^[89,98]. Leys encourage the restoration of earthworm populations by no-till management for the duration of the ley, increased C inputs from roots compared to an arable crop, and increased humus and detritus from ley litter, providing food and habitat^[65,75]. Under herb and grass leys, populations of the anecic earthworm *Lumbricus terrestris* in degraded soils in Germany experienced a rapid increase over 1–2 years, but did not increase further when the duration of ley cropping was extended to 2–3 years^[89]. Prendergast-Miller et al.^[98] corroborate this; earthworm recovery and abundance in a degraded arable soil in England was found to improve under a 2-year grass-clover ley (732 ± 244 earthworms m^{-2}) and this was four times higher than the arable control (185 ± 132 earthworms m^{-2}) which could potentially exceed the permanent grassland earthworm populations (619 ± 355 earthworms $m^{-2}\cdot yr^{-1}$) in field margin soils. Similarly, within a 6-year arable-ley rotation, earthworm biomass under a 3-year simple grass ley reached $187 g\cdot m^{-2}$ compared to $62 g\cdot m^{-2}$ and $30 g\cdot m^{-2}$ under temporary and permanent arable crop, respectively^[99]. This was estimated as an increase of $40\text{--}45 g\cdot m^{-2}\cdot yr^{-1}$ under ley, indicating that within 4–5 years the earthworm biomass could reach that of a permanent grassland, although sometimes this recovery can happen sooner^[99]. Earthworms are crucial engineers of soil structure, improving soil porosity through burrows, reducing bulk density, increasing SOM and redistributing AMF spores and mycelium through grazing^[93,100]. Similarly, within 1 year of establishment of a grass-clover ley, Hallam et al.^[97] reported a decrease in soil bulk density of 6% and an increase in SOM by 9% due to increased earthworm populations.

Although the influence of livestock on soil quality has been well explored in pasture systems (e.g., Abdalla et al.^[101], Stavi et al.^[102]), there is relatively limited information available for the influence of grazed leys on soil structure and key biological indicators of soil quality when incorporated into arable rotations, for example, microbial and fungal community composition or earthworm activity. This is important as arable soils depleted of SOM may be structurally weak, so could be less resilient to the effects of poaching and trampling by livestock when soils are moist. Studies on arable-ley rotations instead focus on soil C and N cycling of ungrazed or cattle grazed leys, discussed in detail in the next section; with limited attention given to the role of sheep grazed leys on soil structure in comparison to those grazed by cattle. The previously discussed studies in this section detailing the influence of ungrazed leys on earthworms, AMF and aggregate stability fail to consider the potential of compaction and excreta returns from grazing livestock. Inclusion of livestock on leys can stimulate increases in earthworm population, soil macrofauna (e.g., dung beetles), microbial and fungal biomass, and above- and below-ground plant biomass, but risk increased topsoil compaction^[71,103].

Partial improvements in soil physical properties have been observed in cattle-grazed integrated crop-livestock systems within the first annual crop cycle after the overwinter ley or cover crop has been reverted back to arable^[104]. Integrated crop-livestock systems typically utilize winter cover-crop grazing by cattle in arable rotations to provide green manure to the soil, control arable weeds and stimulate bioturbation of the soil^[83,105,106]. These winter cover-crops are often plowed into the soil in spring to prepare for the following crop. However, cattle-grazed integrated crop-livestock systems often suffer from increased topsoil (0–5 cm) compaction from livestock trampling of the already weakened arable soil structure^[104]. Static pressure exerted from sheep and cattle hooves averages 66 and 138 kPa, respectively, whereas nominal tire pressures of farm machinery range from 74 to 81 kPa^[107]. This can collapse macropores in the soil surface, reduce soil porosity and hydraulic connectivity, thus reducing water infiltration and promoting surface runoff and flooding^[71]. Soils with high moisture content are vulnerable to collapse and deformation under livestock trampling, leading to soil poaching and erosion^[107,108]. Farmers reintroducing livestock into arable rotations need to avoid overstocking and grazing livestock on weakly structured and fine-textured soils, especially when saturated, to preserve soil structure. An increase in compaction and penetration resistance can have persistent effects on the following crop, reducing root growth and thus yield^[71]. It has therefore been suggested that preference when grazing should be given to sheep over cattle due to lower static pressures and

smaller hoof sizes. In an integrated crop-livestock system in New Zealand, Laurenson and Houlbrooke^[108] found that the soil bulk density under sheep grazing of overwinter forage crops was $1.26 \text{ Mg}\cdot\text{m}^{-3}$ (0–5 cm) compared to $1.35 \text{ Mg}\cdot\text{m}^{-3}$ (0–5 cm) for cattle grazing.

In arable-ley rotations, sheep grazing of a 3–4-year grass-clover ley resulted in increased bulk density in the 0–35 cm layer, reduced air permeability and macropore continuity compared to ungrazed undersown oats^[109]. However, since grass-clover leys have a particularly dense root system, macroporosity (pores > 100 μm) was greater in the 0–10 cm layer than in the undersown oats^[109]. Conversely, Riley et al.^[92] examined soil physical properties within a 15-year mixed dairy-arable field trial under different grazing intensities and management (e.g., organic vs conventional, and different durations of grass-clover ley) on a loam soil in Norway. They found that soil bulk density increased by $0.14 \text{ Mg}\cdot\text{m}^{-3}$ for the continuous arable, but in the cattle-grazed grass-clover ley undersown with cereals in a 4-year rotation, bulk density decreased by $0.03 \text{ Mg}\cdot\text{m}^{-3}$ for a 2-year ley in a standard dairy production rotation and by $0.02 \text{ Mg}\cdot\text{m}^{-3}$ for an organic rotation with a 3-year ley. This was a relatively small reduction in bulk density for both systems with a ley, but may be attributed to post-ley reversion (e.g., plowing) and duration of ley within the 15-year trial. Similar findings were also found for soil porosity and aggregate stability of cattle grazed leys. Soil porosity within continuous arable rotations decreased by 4.3%, but increased by 1.4% for the conventional dairy system with a 2-year ley in rotation and by 0.2% in the organic dairy system with a 3-year ley in rotation^[92]. Notably, continuous arable soils under reduced tillage management had the same aggregate stability as the rotation with a 3-year ley, highlighting the importance of preserving aggregate stability through reduced tillage and reduced compaction^[92,109].

Currently, there are no reported studies that have compared continuous arable cultivation with mown, cattle or sheep grazed leys in rotation under the same soil type, sown botanical composition or different tillage management regimes. However, maintaining the same botanical composition under different sward management in future research may be difficult due to differences in grazing pressure and selective grazing between sheep and cattle; this may impact the persistence of certain plant species in the mixture and thus affect subsequent ley species composition. The influence of both sheep grazing, and in particular, complex multispecies leys, on soil structure and associated biological functions requires further research to critically evaluate the potential benefits and disbenefits sheep grazed arable-ley rotations can provide.

4 CARBON AND NITROGEN CYCLING IN ARABLE-LEY ROTATIONS

4.1 Changes in microbial communities

Arable-ley rotations can alter the microbial community composition, biomass, and activity of agricultural soils and the subsequent cycling of C and N^[110]. Soil temperature, soil properties (e.g., pH), climate, botanical composition of the ley, nutrient and cultivation management can influence the soil microbial community and conversely C sequestration of arable soils^[99,110–112]. Formation of SOC stocks is regulated by the decomposition of SOM and root exudates by Gram-positive and Gram-negative bacteria and saprotrophic fungi^[113]. However, microbial communities responsible for the decomposition of SOC in arable soils are sensitive to temperature changes. With global temperature increases projected to exceed 2 °C under different climate scenarios, this could affect the breakdown of SOC stocks and undermine efforts for C sequestration^[114]. Microbial carbon use efficiency (CUE) determines the allocation of C for biomass growth, respiration (CO₂ emissions), and ultimately necromass^[115]. In a study by Bölscher et al.^[115], sensitivity of CUE to increases in temperature reduced SOC stocks in Swedish grassland soils by 0.1–0.18 $\text{kg}\cdot\text{m}^{-2}$ C, 4% of their current stocks. Unlike grassland, forest or ley farming soils, microbial CUE in continuously cropped soils was not sensitive to temperature changes between 5–20 °C^[115].

Although there has been extensive research on the effects of grassland^[116,117] or arable farming^[118] on soil microbial communities and functioning, there have been few attempts to measure this in arable-ley rotations^[119]. Limited studies, however, have revealed that ley farming can increase the soil microbial biomass^[50,120]. Long-term research on a sandy loam soil in a temperate climate at the Rothamsted Woburn Arable-Ley field trial in England also found that microbial biomass C and N pool was significantly larger in the arable-ley soil ($964 \text{ kg}\cdot\text{ha}^{-1}$ C and $122 \text{ kg}\cdot\text{ha}^{-1}$ N) than the continuous arable control ($518 \text{ kg}\cdot\text{ha}^{-1}$ C and $92 \text{ kg}\cdot\text{ha}^{-1}$ N) following an 8-year fertilized grass ley^[112]. Under different conditions, results from a 25-year arable-ley cropping system experiment in Norway on a silty-sand loam soil in a humid continental climate indicated that there was no increase in microbial species richness or diversity, despite increasing microbial biomass^[121]. The leys in both these studies used either grass or grass-clover leys, with low species diversity. Inclusion of herbal plants, such as plantain (*Plantago lanceolata*) or caraway (*Carum carvi*), into grass-clover mixtures were found to increase the ratio of fungi to bacteria but decrease Gram-positive bacteria, indicating a

faster growing and more active microbial community^[25]. However, as far as it known, there have been no studies on microbial community composition, biomass or diversity within multispecies leys under field conditions.

4.2 Carbon sequestration using arable leys

The *4-per-mille* initiative aims to increase global SOM content by 0.4% per year to compensate for increases in atmospheric GHG emissions^[122]. In particular, the initiative has promoted arable-ley rotations to increase global SOC stocks. In the UK, uptake of arable-ley rotations is projected to increase UK net SOC stocks by 1.6 t·ha⁻¹·yr⁻¹ C, with England in particular accumulating 0.20 t·ha⁻¹ C annually in the 0–23 cm layer^[122,123]. However, the *4-per-mille* initiative has attracted criticism as being impractical and uneconomical in practice for land managers^[124]. This largely depends on the payments system and AES employed on-farm, as under certain schemes, such as the Environmental Land Management scheme in England, this may become economically viable^[125]. The use of arable-ley rotations to increase SOC is highly dependent on soil conditions and management; the limited evidence from long-term replicated trials and measurements of SOC post-ley conversion back to arable requires SOC results to be interpreted with caution. In addition, most studies have focused on a single ley rotation, rather than repeated cycles of multispecies leys and arable cropping. Table 1 presents changes in SOC content reported from previous global field trials utilizing arable-ley rotations; studies relying solely on modeling changes in SOC were not considered.

The information summarized in Table 1 highlights the lack of homogeneity in reporting changes in SOC in arable-ley rotations in the literature. These studies report SOC content to different depths, different units (e.g., weight vs volume basis), and often without reporting the annual C input from the ley in rotation. Further, the lack of bulk density information prevents changes being expressed on a land area basis. Where livestock are used, such as in Clement and Williams^[131], van Eekeren et al.^[99], Chan et al.^[128] and Johnston et al.^[22], the authors did not estimate nutrient recycling from grazing livestock to allow comparison between studies.

Studies on arable-ley rotations (Table 1) have mainly utilized grass or grass-clover leys, with limited attention given to multispecies leys with deep rooted plants that can provide other desirable traits (e.g., pollinator potential and biological nitrification inhibitor production). Botanical composition of the leys is crucial, as inclusion of legumes in grass-clover mixtures provide greater SOC increases than grass-only

leys^[86,132]. Diverse multispecies leys can sequester more C than simple grass or grass-clover leys due to the greater root mass and rooting depth. In New Zealand, diverse swards including herbs such as chicory, plantain and lucerne increased root mass by 5.32–9.35 t·ha⁻¹ compared to 3.81–5.70 t·ha⁻¹ root mass of a ryegrass-clover pasture, thus increasing C inputs into the topsoil (0–30 cm) by 1.20 t·ha⁻¹·yr⁻¹ C^[56]. In comparison, annual soil C inputs from wheat (*Triticum aestivum*) roots were estimated to be only 0.40 t·ha⁻¹·yr⁻¹ C^[133]. Although topsoil SOC is recognized as the most important functionally, the grass only and grass-legume leys listed in Table 1 were reported to suffer from C stratification and limited subsoil SOC increases, attributed to the shallower root depths in common grass-clover leys^[127,131].

Management of leys is also an important factor in C sequestration. Unsurprisingly, SOC content in the topsoil increased with the duration of the ley and the proportion of pasture in the crop rotation^[128]. Grazed leys were found to sequester more C than mowing and removal of biomass as livestock return about a quarter of the OM they consume to the soil increasing SOM content^[86,131]. Livestock excreta inputs and trampling of OM (e.g., plant litter and fecal matter) into the topsoil can increase SOC content by 0.23% compared to 0.17% for mown swards^[131]. This was also seen in Johnston et al.^[22], where SOC content within a 28-year sheep grazed ley rotation increased by 0.33 t·ha⁻¹·yr⁻¹ SOC, equivalent to an increase of 0.9% per year, exceeding the 0.4% increase encouraged by the *4-per-mille* initiative.

The Highfield arable-ley experiment established in 1949 at Rothamsted Research Harpenden, UK, revealed that conversion of grassland to arable decreased SOC stocks by 30%, whereas arable to grassland conversion only increased SOC stocks by 8%^[134]. These changes progressively slowed and tended toward new equilibria, indicating that rates of SOC loss and gain are greatest in the early years of management change. Evidence from long-term field trials, such as those held at Rothamsted Research, provides a warning that SOC content of soils under arable-ley rotations will eventually reach a new equilibrium or quasi-equilibrium where the total SOC will not change but the proportion of SOC in each pool will shift^[22]. Once the new equilibrium is reached, tillage practices such as subsoil ripping may be useful to mix C rich topsoil with the subsoil as a form of occasional tillage where mixing from soil biota and fauna (e.g., earthworms) is not sufficient, allowing for C burial in the subsoil and establishment of a new equilibrium. Concerns over economic losses from the uptake of arable-ley rotations can be mitigated by utilizing livestock on the ley^[135]. However, this would require significant changes in

Table 1 Changes in soil organic carbon (SOC) content in arable-ley rotations, adapted from Schut et al. [24]

Publication	Country	Average annual rainfall (mm)	Soil type	Arable-ley rotation	Ley composition	Ley management	Soil depth measured (cm)	Annual C input to soil	Change in SOC	Comments
Börjesson et al. [126]	Sweden	569 (Lönnpstorp), 558 (Lanna)	Loam (Lönnpstorp), clay (Lanna)	4-year rotation: 3 years ley, 1-year cereal crop	Grass-clover (meadow fescue, timothy, and red clover)	Mown with biomass removed. Four N fertilizer treatments: 0, 50, 100, and 150 kg-ha ⁻¹ -yr ⁻¹ N	0–20	1.7–2.5 t-ha ⁻¹ C in leys, 0.25–1 t-ha ⁻¹ C in cereals	+0.28 (-N) + 0.35 (+N) t-ha ⁻¹ C at Lönnpstorp. +0.07 (-N) and +0.17 (+N) t-ha ⁻¹ C at Lanna	No significant effect of N fertilizer on SOC stocks in the ley rotations
Johnston et al. [127]	UK	640	Sandy loam	Alternating rotation lengths followed by 8-year ley rotation. All leys followed by 2-year arable crop	Grass or grass-clover (ley composition not given)	Grazed by sheep or mown with biomass removed	0–25	n.d.	+0.01 t-ha ⁻¹ C for 3-year grass ley. +0.16 t-ha ⁻¹ C for 3-year grass-clover ley. +0.36 t-ha ⁻¹ C for 8-year grass ley. +0.28 t-ha ⁻¹ C for 8-year grass-clover ley	Long-term field trial started in 1938. Changes in SOC data are measured from 1965–1974 to 2000–2009
Krauss et al. [127]	Switzerland	1303 (2012), 1112 (2013), 966 (2014)	Calcareous clay	6-year rotation: 2-year ley, 4-year arable crop	Grass-clover (ley composition not given)	Mown with biomass removed. Cattle slurry applied after each cut	0–50	n.d.	+8.1 t-ha ⁻¹ C for arable-ley soils under reduced tillage and manure application compared to manure applications, with highest SOC content in conventional tillage	Increased stratification in soils under no-till management with application compared to manure applications, with highest SOC content in the surface soil. SOC did not increase in lower layers
Albizua et al. [50]	Sweden	655	Sandy loam, sandy clay loam, and coarse-loamy soil	4-year rotation: 1-year ley, 3-year crop	Grass (ley composition not given)	Mineral N fertilizer addition: 0–150 kg-ha ⁻¹ N. 20–30 t-ha ⁻¹ of manure applied following wheat harvest at the end of the 4th year in rotation	0–20	n.d.	+0.39% SOC in 0 kg-ha ⁻¹ N ley system + 0.1% SOC in 150 kg-ha ⁻¹ N ley system	Leys in rotation with additional mineral N fertilizer inputs results in positive effect on SOC content
Chan et al. [128]	Australia	544	Clay loam	3- or 6-year rotation: see paper for more details	Grass-legume (reed canary grass, cocksfoot, lucerne, and subterranean clover) or grass-clover (annual ryegrass and subterranean clover)	Grazed by sheep or mown with biomass removed	0–30	n.d.	+500–700 kg-ha ⁻¹ -yr ⁻¹ C	Long-term field trials ranging 13–25 years. Increased SOC stocks following ley are quickly depleted by tillage and crop residue management
Bolinder et al. [129]	Sweden	567 (Offer), 490 (Ås), and 566 (Röbäcksdalen)	Silty clay loam	6-year rotation: 1–5 years of ley and arable crop	Grass-clover (meadow fescue, timothy, and red clover)	Ungrazed. Manure applied to ley in autumn (20 t-ha ⁻¹)	0–25	n.d.	+12 g m ⁻² -yr ⁻¹ C for rotation A (5 years ley, 1-year crop)	60-year field-trial of leys in organic dairy cropping systems

(Continued)

Publication	Country	Average annual rainfall (mm)	Soil type	Arable-ley rotation	Ley composition	Ley management	Soil depth measured (cm)	Annual C input to soil	Change in SOC	Comments
Chirinda et al. ^[130]	Denmark	704	Sandy loam	4-year rotation: 1-year ley, 3-year arable with catch crops or red clover and undersown in the perennial ryegrass barley crop	Grass-clover (white clover and perennial ryegrass or red clover and perennial ryegrass)	Mown with biomass removed. Anaerobically digested pig slurry used as N fertilizer	0–30	3.95 ± 0.06 t-ha ⁻¹ C between 1997 and 2007	-1 g.kg ⁻¹ C between 1996 and 2008	10-year field trial. Increased C inputs resulted in increased microbial activity but not C storage
Christensen et al. ^[86]	Denmark	862	Sandy loam	6-year rotation: 1–6-year ley in rotation with barley undersown with grass	Grass (perennial ryegrass, meadow fescue, timothy, smooth meadow grass)	Mown with biomass removed. 225 kg-ha ⁻¹ yr ⁻¹ N of mineral fertilizer applied. 75 kg-ha ⁻¹ N after the first and 50 kg-ha ⁻¹ N second cut	0–20	n.d.	+ 1100 kg-ha ⁻¹ yr ⁻¹ C in soils under ley	Lack of residual effect highlights need for legumes in the ley composition
van Eekeren et al. ^[99]	Germany	n.d.	Sandy loam	6-year rotation: 3-year grass-clover ley, 3-years arable crop	Grass-clover (perennial ryegrass and white clover)	Grazed by dairy heifers from 1966 to 1989. Mineral N fertilizer: either 0 or 354 kg-ha ⁻¹ yr ⁻¹ N	0–10	n.d.	+ 7.5 g SOC kg ⁻¹ dry soil	Long-term field trial established in 1966 (* SOC content of permanent arable minus average SOC of temporary grassland and temporary arable)
Studdert et al. ^[96]	Argentina	870	Loam	7-year rotation: 2-year or 4–5-year ley with respective crops in rotation	Grass-legume (cocksfoot, bulbous canary grass, tall fescue, perennial ryegrass, white clover and alfalfa)	Mown with biomass removed	0–15	n.d.	Returned to original SOC content 3–4 years under ley (37.2 g SOC per kg)	Long-term field trial established in 1976–1993
Clement and Williams ^[131]	UK	n.d.	n.d.	4-year ley	Grass-clover (see paper for details)	Grazed and mown for hay, with the post-mown ley grazed	0–15	n.d.	+ 0.23% SOC for grazed ley; + 0.17% SOC for cut and then grazed ley	Increases in SOC was limited to 2–3 cm of the topsoil

Note: n.d., not determined; SOC, soil organic carbon; +, increases in SOC; -, decreases in SOC; and +N or -N with or without mineral N fertilizer.

Plant species scientific names: Bulbous canary-grass (*Phalaris aquatica*), cocksfoot (*Dactylis glomerata*), lucerne or alfalfa (*Medicago sativa*), meadow fescue (*Festuca pratensis*), meadow grass (*Poa annua*), red clover (*Trifolium pratense*), reed canary grass (*Phalaris arundinacea*), ryegrass (*Lolium perenne*), subterranean clover (*Trifolium subterraneum*), timothy (*Phleum pratense*), white clover (*Trifolium repens*).

policy, governance and stewardship schemes to encourage arable farmers to diversify their enterprise or engage with partnerships at a community level and establish grazing agreements with livestock farmers.

4.3 Biological N fixation and nitrification inhibitors

In most arable agriculture, mineral N fertilizers have largely replaced the use of legumes for increasing N content of soils^[136]. However, restrictions on fertilizer usage to constrain water and air pollution, consumer demand for organically grown produce, and increases in mineral fertilizer costs has returned attention to legumes in cropping rotations^[136]. The symbiotic relationship between legumes and rhizobacteria (such as *Rhizobium* or *Bradyrhizobium*) within root nodules allows for atmospheric N₂ fixation in return for plant generated carbohydrates^[59]. This can also encourage the growth of grasses, leading to greater SOC inputs. For a 16-species diverse plant species mix, soil C and N increased by $70 \pm 9 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ C and $3.5 \pm 0.53 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ N, respectively, compared to monocultures of the same species ($14 \pm 10 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ C and $0.59 \pm 0.57 \text{ g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$ N)^[137]. Due to their N fixing abilities, legumes in leys can be used as a partial or full replacement for mineral fertilizers. Depending on abiotic conditions in the year of establishment, grass-clover leys plowed into the arable rotation saved 50%–83% or 77%–92% of fertilizer N typically applied during the arable phase of the rotation^[31]. However, while grass-clover leys can reduce mineral fertilizer N inputs, plowing risks N leaching and subsequent eutrophication and nitrate (NO₃⁻) pollution of watercourses^[61], as well as release of the potent greenhouse gas N₂O. Under min-till management, these N losses may be greatly reduced.

Nitrification inhibitors (NIs) are used in agriculture to improve nitrogen use efficiency (NUE) by inhibiting the bacterial oxidation of ammonium (NH₄⁺) to NO₃⁻, thus reducing N losses through leaching, runoff and denitrification^[138]. Synthetic NIs such as DMPP (3,4-dimethylpyrazole phosphate) and DCD (dicyandiamide) are often used, however, these have varying levels of effectiveness and risk leaching into the environment and the contamination of water sources^[139,140], and in the case of DCD, potential contamination of the food-chain^[141]. Effectiveness of NIs is highly dependent on soil properties (e.g., pH and texture), crop type (e.g., cereals vs forage crops) and management factors (e.g., N fertilizer rate, and irrigation vs rainfed crops)^[142]. When applied to cattle urine, DCD was effective at reducing N₂O emissions by 70% but not NO₃⁻ leaching or ammonia (NH₃) emissions^[143] whereas DMPP was ineffective at reducing N₂O emissions

from cattle and sheep urine patches^[143,144].

Naturally occurring NIs, or biological nitrification inhibitors (BNIs), are excreted from root exudates by herbs such as plantain to inhibit nitrification in the surrounding soil in N limited environments^[145]. Plantain contains high levels of plant secondary metabolites such as aucubin, acteoside and catapol that act as BNIs of enzymes and nitrifying bacteria responsible for nitrification, *Nitrosomonas* and *Nitrobacter*^[146–148]. A laboratory incubation study by Dietz et al.^[146] reported that the addition of plantain leaves resulted in reduced soil NO₃⁻ content for the 56 days in incubation. However, these plant secondary metabolites vary with growing season and climate. Aucubin concentrations in plantain increased from 3.8 to 6.9 mg·g⁻¹ DM in the first and second growing season^[149]. This can have implications for its efficacy as a BNI and the potential effect on NUE and N partitioning in livestock. Livestock grazing leys containing plantain can excrete plant secondary metabolites such as aucubin into the soil in urine, suppressing microbial activity within the urine patch^[147]. The effect of plant secondary metabolites on livestock is explored in detail in the following section.

4.4 Enteric CH₄ emissions

Ruminants, such as cattle and sheep, represent significant sources of CH₄ emissions in livestock production. In 2017, cattle and sheep in the UK produced 16.8 and 4.0 Mt CO₂e of CH₄ by enteric fermentation^[150]. CH₄ is a powerful GHG, with a lifetime of 12.4 years and 100-year global warming potential (GWP) 28–36 times that of CO₂^[151] and a 20-year GWP 86 times that of CO₂^[152]. Recent revisions to GWP calculations have led to the development of GWP*, which accurately accounts for the reduced radiative forcing of short-lived climate pollutants such as CH₄ compared to long-lived climate pollutants such as N₂O and CO₂^[153]. GWP* is currently used to increase the modeling accuracy assessing mitigation measures to reduce the future impacts of ruminant production, helping countries identify feasible methods to achieve the coveted Net Zero C emissions^[152]. In ruminants, CH₄ production can vary with diet, animal, rumen microbiome composition and health. Currently, the IPCC default tier 1 emission factor estimates enteric fermentation emissions from sheep in developed countries as 8 kg CH₄ per head per year and requires refining^[154].

The use of plant secondary metabolites for enteric CH₄ mitigation in ruminants has been extensively reviewed in the literature^[155–158]. Key herbs used in multispecies leys such as chicory, plantain, sainfoin and birdsfoot trefoil (*Lotus*

corniculatus) contain plant secondary metabolites such as tannins, saponins and essential oils that can moderate the microbial production of CH₄ in the rumen^[159]. Saponins can suppress dihydrogen producing rumen protozoa, essential for the production of CH₄^[160]. Similarly, condensed or hydrolyzable tannins can reduce CH₄ production by preventing fiber degradation in the rumen by complexing with proteins that are released for degradation in the low pH of the abomasum^[158,161]. Addition of tannins and saponins as a feed supplement, however, should be used with caution, as hydrolyzable tannins and saponins in the rumen can be toxic to the host animal as well as to methanogens^[157,160,162].

Naturally occurring tannins in chicory and sainfoin vary in concentration according to genotype, season, and management. Consequently, making measurements of tannin content within the forage is crucial for studies when investigating changes in rumen CH₄ production. Addition of tanniniferous crop species to ensiled or dried forages (e.g., hay) has recently been found to decrease CH₄ emissions^[163]. In contrast, tannin addition to beef cattle fed a basal diet of lucerne and barley (*Hordeum vulgare*) silage reduced rumen ammonia concentration but not daily CH₄ production^[162]. Similarly, no differences in daily CH₄ emissions were found for cattle fed sainfoin and birdsfoot trefoil hay^[164]. This was attributed to the drying of plants inactivating the bioactive tannins in the forage^[164]. However, enteric CH₄ emissions were reduced from sheep fed either ensiled mixes of timothy (*Phleum pratense*) with either sainfoin (29.7 g CH₄ kg⁻¹ DM intake) or red clover (*Trifolium pratense*) (30.5 g CH₄ kg⁻¹ DM intake) containing high levels of condensed tannins and polyphenol oxidase respectively, compared to pure ensiled timothy (35.7 g CH₄ kg⁻¹ DM intake)^[165]. In fresh forage, no differences in daily CH₄ production was observed in sheep fed fresh chicory or ryegrass, with 24.1 and 21.4 g CH₄ kg⁻¹ DM, respectively^[166]. For sheep fed ryegrass or a multispecies mix containing clover and herbs (herb composition was unspecified), CH₄ production was lower in the multispecies mix (16.1 g·d⁻¹ ryegrass vs 12.9 g·d⁻¹ multispecies)^[167].

The variable reports in the literature of plant secondary metabolites reducing CH₄ emissions within livestock systems indicates that much more work needs to be undertaken to explore the complex relationships between diet and CH₄ production. The effect of grazing ruminants on multispecies leys with herbs containing plant secondary metabolites is relatively understudied but is thought to affect other aspects of the ruminant, such as N excretion and parasite burden^[168]. This indicates that a single focus on CH₄ and live weight gain also needs to be coupled with studies of other aspects of rumen functioning.

4.5 Urine-patch N₂O emissions

Increasing the available grazing area in the UK by reintroducing grazed leys into arable rotations risks increasing livestock N₂O emissions. As ruminants are relatively inefficient at N assimilation, only 5%–10% of the N consumed is utilized in meat, milk and wool production, with the remainder excreted in urine and dung^[169]. Urine deposited to pasture contains 70%–75% and 45%–60% of N excreted by sheep and cattle, respectively, and represent significant sources of livestock N₂O emissions^[170]. N₂O is a potent GHG, with a GWP 298 times that of CO₂ that requires careful monitoring^[171]. Between 1961 and 2014, 54% of global annual N₂O emissions from grasslands were attributed to livestock excreta deposits, with only 13% and 7% attributed to manure N and mineral N respectively^[172]. Urine in particular contains readily available C and N that produce hotspots of N₂O emissions within the grazing pasture, whereas dung contains more insoluble forms of N thus is more inert and slower to breakdown into N₂O^[171,173,174].

Sheep urine composition and N content is heavily dependent on diet and animal health, and ranges from 1.2 to 13.0 g·L⁻¹ N^[175]. 25%–90% of N in urine consists of urea, followed by purine derivatives and non-urea compounds: hippuric acid, allantoin, creatine, creatinine, uric acid, xanthine and hypoxanthine as well as any plant secondary metabolites such as the aucubin derivative aucubigenin^[169,176–178]. Diet manipulation, for example, by introducing plants with particular secondary metabolites into the pasture, can have a diuretic effect, reducing the proportion of urea in the urine and increasing the content of less labile non-urea compounds^[178]. This has potential for use in swards containing high clover content to increase productivity and milk production, but exceeds the animal's requirement for N resulting in increased N excretion to pasture^[176]. Plant secondary metabolites, such as tannins, can also increase the proportion of N in dung, which is less vulnerable to N₂O and NH₃ losses than urine^[173,179]. However, the effect of plant secondary metabolite containing plants in temperate multispecies leys on ruminant urine composition and subsequent N₂O emissions is relatively understudied. A recent meta-analysis found that the relationship between animal diet and urine composition were under-reported in the literature^[170]. Currently, the literature available for urine-patch N₂O emissions, and subsequent emission factors, is dominated by grass or grass-clover pastures with almost no information available for multispecies leys.

In 2019, the IPCC announced the refinement of the 2006 Guidelines for National Greenhouse Gas Inventories, including refinements for the N₂O emission factor for livestock urine and

dung on pasture, range and paddock (EF_{3PRP}) for sheep and cattle^[180]. This reduced the 2006 EF_{3PRP} from 1% to 0.3% of the N applied to the soil in urine and dung emitted as N₂O^[154,180]. There are currently no studies used by the IPCC to refine the EF_{3PRP} that use emissions reported from grazed herbs such as chicory. Although some studies did not report sward composition^[181–183], calculations were made from predominantly grass swards^[184–192], grass-clover^[144,186,193–198] or forage crops (e.g., barley, lucerne, or brassicas such as rape, *Brassica napus*, or kale, *Brassica oleracea* var. *sabellica*)^[186,194,199]. Currently, no estimates of direct livestock excreta N₂O emissions from grazed multispecies pastures or leys are included in the IPCC calculations.

Although diet can alter the ratio of N in urine and dung, key soil properties such as pH, moisture, porosity, temperature, texture and microbial activity can affect N cycling in the urine patch^[4,148,170,200]. Microbial activity responsible for N₂O emissions by the processes of nitrification (NH₄⁺ to NO₂⁻ then to N₂O and NO₃⁻) and denitrification (NO₃⁻ and NO₂ to N₂O, NO_x and N₂) can be altered by plant secondary metabolites within a multispecies ley^[178,201]. Urine-derived plant secondary metabolites such as acteoside, aucubin and isothiocyanates can act as natural NIs within the urine patch, suppressing the microbial activity of nitrate-oxidizing bacteria, ammonia-oxidizing archaea and ammonia-oxidizing bacteria^[149,178,202]. However, as with urine composition, the plant secondary metabolite content of urine and longevity in the soil is also inadequately studied. It is unclear what form plant secondary metabolites exist as when broken down in urine or how much is excreted. For a dairy system, Gardiner et al.^[147] estimated a potential aucubin urine excretion rate of 0.49–3.40 t·ha⁻¹ for cattle grazing pastures with variable proportions of plantain in the sward composition. Further, the persistence of these plant secondary metabolites in the subsequent crop remains unknown.

Several recent studies have investigated the effect of multispecies swards on N₂O losses, particularly in dairy production systems. Di et al.^[194] reported a reduction of ~30% in cattle urine N₂O emissions from lysimeters from lucerne grazed pasture compared to a ryegrass-white clover pasture, 7.1 and 10.9 kg·ha⁻¹ N₂O-N, respectively. In pastures containing 45% plantain, cattle urine-patch N₂O emissions were observed to decrease from 6.9 to 1.8 mg·m⁻²·h⁻¹ N^[169]. This was observed to be a result of reductions in urine-N content from grazing key plants containing high levels of secondary metabolites. Cattle urine-N content was also observed to decrease from 6.1 g·L⁻¹ N for a simple pasture to 4.9 g·L⁻¹ N for a multispecies pasture containing chicory, plantain and

lucerne^[203]. Currently, there are no reports on sheep urine-patch N₂O emissions from grazed multispecies leys under field conditions. Focus in the literature is predominantly on dairy production systems, as multispecies pastures can increase the proportion of N used in milk production in cattle^[204]. This was observed in an indoor feeding trial; dairy cattle fed a multispecies mix containing perennial ryegrass, prairie grass (*Sporobolus cryptandrus*), white clover, chicory, plantain, and lucerne, had a higher milk yield (12.5 vs 11.3 kg·d⁻¹ per cow) and higher percentage of N allocated to milk production (23% vs 15%) compared to cows fed a grass-clover mixture^[205]. Alongside the numerous ecosystem benefits multispecies leys provide, plants containing high levels of secondary metabolites within a multispecies ley may offer a potential mitigation option for livestock agriculture by reducing excreta patch N₂O emissions. However, it is important to note that the sward composition of multispecies leys is crucial, as high proportions of grasses and clovers as well as other herbs with low levels of plant secondary metabolites may dilute the effects of plants with high levels of secondary metabolites and weaken potential benefits. Importantly, the presence of these plants within a multispecies ley does not necessarily mean they are grazed by livestock. The astringency of plants such as chicory often reduces feed intake until livestock adjust to the taste difference or grazing management is changed to encourage consumption, for example, increased stocking density or rotational grazing.

5 LIVESTOCK HEALTH AND PRODUCTIVITY

In addition to reducing livestock GHG emissions, multispecies leys have the potential to improve livestock productivity, particularly in grazing lambs. Due to the implications for soil structure, preference in arable-ley rotations should be given to sheep grazing over cattle. Arable-ley rotations offer the potential for healthier grazing as newly established leys in arable rotations have reduced gastrointestinal nematode burdens than previously grazed permanent pasture^[206]. Gastrointestinal nematodes in livestock systems have significant economic impacts, costing the UK sheep industry 84 million GBP·yr⁻¹^[207]. Since the introduction of the first anthelmintic, phenothiazine, in the 1950s gastrointestinal nematodes in livestock have developed resistance to commonly used anthelmintics^[208,209]. In the UK, increasing resistance to commonly used anthelmintics, such as benzimidazoles, levamisole and macrocyclic lactones, has pushed the UK livestock industry to consider alternative methods of gastrointestinal nematode management^[210]. Gastrointestinal nematodes can pass between untreated animals grazing the

same pasture and survive outside the host in the sward^[206]. Livestock productivity is affected by common gastrointestinal nematodes, such as *Nematodirus* and *Hemonchus contortus*, and results in livestock suffering from anemia, edema, weakness, reduced meat, milk and wool production^[211,212].

Plant secondary metabolites within multispecies pastures are promoted as natural anthelmintics and can reduce parasite burden in livestock^[211]. Studies including plantain, chicory, birdsfoot trefoil and lucerne in multispecies pastures found lower fecal egg counts in infected lambs than comparator grass or grass-clover pastures^[213]. Tannins in multispecies pastures can decrease motor activity of gastrointestinal nematodes, inhibit the transformation of eggs to larvae, and inhibit the energy metabolism of gastrointestinal nematodes^[214]. A study investigating plantain, chicory and grass swards versus permanent grass pasture found no differences in final fecal egg counts^[215]. However, sparse and upright stems within the sward architecture in the plantain-chicory pasture was attributed to reduced adult (L3 parasite stage) parasite populations^[215]. It is important to note that including plants with high levels of plant secondary metabolites in swards may not alleviate preexisting high-level gastrointestinal nematode burdens and should not replace effective pharmaceutical anthelmintic treatment on farms, but may reduce the frequency of anthelmintic use and increase the time between treatments. This was demonstrated by Grace et al.^[35], where lambs grazing a nine-species multispecies sward required their second anthelmintic dose 59 days after the first treatment compared to lambs grazing a perennial ryegrass sward which needed another dose after 36 days.

As well as reducing parasite burdens, multispecies pastures can also increase live weight gains in livestock. Increased crude protein content in multispecies pastures can increase muscle mass and milk production, increasing livestock performance^[216,217]. In New Zealand, lambs grazing a herb-clover pasture experienced weight gains of 0.4 kg·d⁻¹^[218]. Similarly, lambs grazing a grass-clover sward had a slower live weight gain and lighter carcass weight per ha over three years (1.27 t·ha⁻¹) compared lambs grazing a multispecies mix of either red and white clover with plantain (1.71 t·ha⁻¹) or chicory and plantain mixture (1.73 t·ha⁻¹)^[219]. Reducing the time it takes to reach slaughter weight has, having implications for the carbon footprint and life cycle assessment of lowland meat production. In Ireland, lambs grazing a six- or nine-species pasture containing grasses, legumes and herbs were found to take 168 days to reach slaughter weight compared to lambs grazing pure grass pastures, which took 181 days^[35]. Currently, the majority of published studies investigate the

impact of an individual herb or legume species, e.g., chicory or sainfoin, on livestock productivity or livestock health. However, there is minimal evidence available for the benefits of multispecies leys on livestock productivity. Future studies should carefully consider sward composition in their experimental design, as multispecies ley mixtures vary between seed companies, ranging from four plant species selected from each plant group (grasses, legumes and herbs) to 16 plant species. Mixtures containing a higher diversity could potentially dilute the effect of plants containing high levels of secondary metabolites within the sward, negating their full potential and resulting in variable results across studies.

6 SUSTAINABLE AGRICULTURAL INTENSIFICATION AND RESILIENCE

Achieving sustainable agricultural intensification is a key cornerstone of environmental research. Arable-ley rotations have long been recognized for their potential for sustainable intensification, with evidence of increases in biodiversity^[50], yield^[220], soil nutrients (e.g., N) and organic matter^[119], improvements in soil structure^[97] and in ungrazed rotations, subsequent crop performance including under drought and flood stresses^[65]. The recycling of nutrients and organic matter from livestock grazing has been observed to increase crop yields in integrated crop-livestock systems, but there is minimal data available for crop yields after the reversion of livestock-grazed leys back to arable. In a cattle grazed integrated crop-livestock system, dung inputs increased the availability of soil K and P by 122% and 38%, respectively, subsequently increasing the yield and number of pods per plant of the following soybean crop by 23% and 20% relative to the ungrazed control^[83]. However, Taylor et al.^[220] found that following a 3-year grass-clover ley grazed by sheep in Scotland, cereal crop yield was highest in the first year following ley reversion back to arable than the second year, producing in the first year and the second year 5.06 and 3.45 t·ha⁻¹ of grain and 3.60 t·ha⁻¹ and 2.26 t·ha⁻¹ of straw, respectively, demonstrating that potential increases in yield are relatively short-lived.

Due to the N fixing capacity of legume containing leys, arable-ley rotations have the potential to reduce the N requirement of the following crop. In the UK, use of mineral N fertilizer has increased by 7.4% between 2008 and 2018, from 0.96 to 1.03 Mt, respectively^[221]. By 2030, global demand for mineral N fertilizer to maintain production is predicted to reach 135 Mt^[4]. The BNF ability of legumes within a multispecies ley could help to reduce the mineral N fertilizer demand of conventional farms. However, there is limited information

available on the effect of leys in rotation on the nitrogen fertilizer replacement value (NFRV) of plowed out leys on crop nitrogen requirements. In Belgium, after a 2-year grass-clover ley was plowed and reverted back to a forage maize (*Zea mays*) crop, the NFRV was highest in the first year at 177 kg-ha⁻¹N but declined successively over the 3-year period, averaging 79 kg-ha⁻¹ N in the second year and 31 kg-ha⁻¹ N in the third^[222]. However, while a reduction in required fertilizer N was observed, Cougnon et al.^[222] noted that ley management (i.e., grazing or mowing) did not affect the NFRV. Currently, there is no estimate of the NFRV potential of multispecies leys in rotation.

As well as their ability to reduce mineral N inputs and increase yield, multispecies leys have greater resilience to environmental stresses and extreme weather events than their grass or grass-clover counterparts. The deep rooting capabilities of key species, for example, chicory, yarrow (*Achillea millefolium*), lucerne and sainfoin, can allow plants greater access to water during drought conditions and maintain biomass production^[217]. This could help to maintain productivity and resilience of the farm enterprise, as most countries are expected to experience more extreme weather events due to climate change; however, little is known about the effect of drought and flood events on multispecies leys under field conditions. At the time of this review, there was no available literature on the effect on microbial activity, yield and C and N cycling on multispecies leys under environmental stresses.

Deep rooted species may have the potential to access micronutrients in the subsoil and bring them to the surface to be made available to grazing livestock and cereal crops for human consumption. Micronutrient deficiencies in arable agriculture is often termed the ‘hidden hunger’, as deficiencies in iodine, iron and zinc content in cereal crops have implications for human health^[223–225]. This is also seen in livestock, as micronutrient deficiencies in the sward composition of grazing pastures can affect the reproductive system in livestock and subsequent meat and milk production, quality, and micronutrient content^[224,226]. Compared to grass pastures, herb-rich pastures containing chicory, plantain, white and red clover were found to have greater micronutrient content concentrations of cobalt, copper, zinc and iron but not molybdenum^[227]. The grass species cocksfoot was found to have the greatest concentration of manganese^[227], however, this response is expected to be highly soil type specific. Pirhofer-Walzl et al.^[228] identified that herbs such as chicory, plantain, caraway and salad burnet (*Sanguisorba minor*) in the multispecies mix had higher levels of sward macro- and micronutrients (e.g., zinc) than grasses and legumes. However,

little is known of the effect of these multispecies leys on the micronutrient content of meat and milk production. If herb-rich leys can increase the micronutrient content of livestock products, it may help to address the hidden hunger in modern food production.

Despite the benefits multispecies leys can provide, it cannot be considered a magic bullet for many of the problems facing arable agriculture today. Under an organic farming scenario, if the UK, for example, was to shift to utilizing arable-ley rotations, GHG emissions and crop yield would reduce as production pressure is shifted overseas^[229]. If arable-ley rotations were used in conventional agriculture as well this may be avoided. However, arable-ley rotations also face socioeconomic barriers to uptake. A scoping study found that commonly cited reasons against utilizing leys were: (1) short-term economic losses, (2) lack of existing partnerships between arable and livestock farmers, (3) lack of skilled workers with animal husbandry skills, and (4) limited evidence of proven benefits for livestock and arable farmers^[135]. A recent review by Schut et al.^[21] highlighted that the socioeconomic limitations to reintroducing arable-ley rotations, and thus the recoupling of integrated crop-livestock system, in the EU was mainly driven by the lack of suitable infrastructure, for example, abattoirs and grazing agreements, to support arable-ley rotations. For farmers to overcome these barriers, a change in infrastructure, increased financial support, and improved evidence base evaluating the potential benefits and consequences of arable-ley rotations are needed to help farmers make informed decisions.

7 SUMMARY

The use of ungrazed leys in arable-ley rotations is shown to increase ecosystem service delivery in agriculture through increasing C sequestration, symbiotic nitrogen fixation, water infiltration, biodiversity in soil fauna and microbial communities. However, currently there is insufficient literature available for arable soil improvement under grazed leys, particularly multispecies leys. The majority of previous research has been conducted on grass or grass-clover leys in arable rotations, resulting in a limited evidence base to support and justify the use of multispecies leys in cropping rotations. This review has highlighted that due to their complexity and complementarity of species, multispecies leys can potentially deliver greater ecosystem services than comparator grass or grass-clover ley. Increasing species diversity by utilizing a four- to eight-species multispecies ley can offer greater multifunctionality and opportunities to improve soil quality

than a monoculture grass or low diversity (e.g., two- to four-species) grass clover ley. This review has examined the available literature and identified key knowledge gaps in the current understanding of grazed arable-ley rotations. These are as follows:

- (1) There is a lack of evidence available on the effect of grazed multispecies leys on AMF, soil biodiversity, soil microbial communities and functioning in degraded arable soils.
- (2) Further research is needed on the effect of plant secondary metabolites in fresh and ensiled multispecies leys on rumen microbiome functioning, livestock enteric CH₄ emissions, livestock NUE, and gastrointestinal parasite burden.
- (3) Lack of information for the biological nitrification inhibitor potential of multispecies leys in urine patches and after mineral N fertilizer applications.
- (4) There is a need to assess if a different N₂O EF_{3PRP} for excreta deposited by livestock grazing multispecies leys is needed for national and international greenhouse gas inventories, as this is lacking in the IPCC calculations and in the available literature. Without an accurate N₂O EF_{3PRP} and enteric methane measurement this reduces the resolution of future carbon footprints and life cycle assessments.
- (5) No information is available for micronutrient sward content of multispecies leys and the subsequent micronutrient content of meat and milk from grazed livestock.
- (6) There is minimal information available on the tolerance and resilience of multispecies leys to extreme weather events (e.g., drought or flood) with respect to species persistence, pasture yield and quality, and ecosystem services.
- (7) Although there is ongoing research across the EU, there is a lack of published data on the effect of reversion from

multispecies leys to arable crop as part of the arable-ley rotation with regards to long-term SOC stocks, soil microbial community functioning, and soil N and C cycling.

- (8) Further research is needed to provide replicated long-term trials (10-25 years) to evaluate greater ecosystem services (e.g., flood reduction) and identify the best method of ley management (i.e., mowing vs grazing, and sheep vs cattle).
- (9) Socioeconomic research is needed to identify cultural barriers and evaluate the economic impacts of multispecies leys and the reintegration of arable-ley rotations to provide an evidence base for on-farm and country-specific economic assessments.

Further research is required to support the development of new policies and legislation to encourage the use of livestock and multispecies leys in arable rotations. Following new research, governments should support provide additional infrastructure (e.g., abattoirs) in predominately arable regions (e.g., eastern Europe) in addition to establishing national grazing networks linking arable and livestock farmers through grazing agreements. This may help overcome the socioeconomic barriers (e.g., skill gaps) that may be limiting the uptake of livestock and leys in arable rotations. Future agri-environment payment schemes should also consider payments for grazing livestock on arable land to encourage a reduction in mineral fertilizer use while improving soil quality. This may help to reduce short-term economic losses often incurred while adopting mixed farming methods. To enable farmers to make informed decisions for what is best for their land, research needs to fill these knowledge gaps and produce evidence-based recommendations.

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Compliance with ethics guidelines

Emily C. Cooleidge, David R. Chadwick, Lydia M. J. Smith, Jonathan R. Leake, and Davey L. Jones declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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