Temporary grassland leys and agricultural soil health

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Abstract

Grasslands are found across most biomes globally, and have been manipulated by humans for millennia. Under modern management, mainly for grazing livestock, grasslands can be established for as little as 1-5 years. They are subject to a variety of inputs, grazing pressures, cover crop species, and durations of establishment. Because of the scale of grassland land cover globally, they are a potential resource for tackling three challenges centred around carbon: soil health, climate change, and food production. Soil carbon is essential for soil health and maintaining the capacity for sustainable harvest, but carbon has been lost from soil to the atmosphere.

Using *in situ* and *ex situ* experiments, and a meta-analysis, we investigate interactions between different cover crop species and their effect on carbon, both total and in pools of recalcitrance, across scales of time and depth. We investigate the potential for short-term leys to improve agricultural soil health by incorporating carbon from the atmosphere, and the processes by which carbon might be transferred back to the soil. There is a focus on depth, to explore the potential for leys to store carbon away from atmospheric and environmental interference, and on time, due to the short-lived nature of grassland leys. Grazing is explored as a potential tool for grassland management for soil emissions reduction.

This work investigates the potential for storing carbon, in fractions of recalcitrance, across depth profiles of soil under temporary grassland leys. Experiments over 1-2 years show time progression of carbon reactivity and how plant species influence total carbon in soil with different management strategies. This thesis demonstrates the complexity of interactions in processes that affect soil carbon, and suggests new avenues for future research into these processes.

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1: Introduction

1.1 Introduction to soil carbon

Carbon is a crucial element in the health and fertility of soils, which underpin all life and play a crucial role in human history and productivity (Kutsch *et al*., 2009). Agriculture, built on soil, is key to the many technological, scientific, social and cultural advances of the last two centuries, from the adoption of mechanisation freeing workers up for urban industries, to the greater nutritional provision increasing health, productivity and life expectancy in the population, to the green revolution increasing global trade (Gollin *et al.,* 2005).

However, the soil base on which we rely is currently facing many issues. It is within this intersection of challenges, ranging from carbon depletion to erosion, explored below, that the contribution of soil carbon must be investigated, through the cyclical relationship of agriculture and soil health. Carbon stored in the soil essentially depends on the residual between inputs and outputs, and the mechanisms which control these are intricately connected (Dignac *et al.,* 2017). Storage of carbon and its relation to soil properties can be viewed through the prisms of i) the plants established in the soil, and what effect they have on both soil characteristics and soil microbiota; ii) through the microbial community, and its relationship between the plant community and the SOC iii) how the soil depth and the time frame influences the storage of carbon and how these affect the form in which carbon is stored or accessed; and iv) the influence of current soil management.

Carbon is the key element in the terrestrial biome, with 500 Pg held in plant biomass and 2000 Pg held in soil organic matter (SOM) (Janzen, 2004). Soils and sediments are the largest store of carbon on the planet after rocks (Johnson *et al*., 2015), with the top metre of the world's soils containing 3x as much C as the atmosphere and 4.5x as much as in the biota (Lal, 2013). Soil is the most active pool in the terrestrial carbon cycle (Janzen, 2004), with the carbon stored within subject to flux in and out under natural processes. Because soil is such a large store of C, its management and the ensuing changes in soil C concentration have a proportionately large influence on global atmospheric C enrichment (Lal, 2013). Carbon in soil is generally grouped into 'active' and 'passive' pools in the established literature; these are relatively poorly defined, but refer to the residence time, the lability, and the sensitivity to change of that carbon pool (Sahoo et al., 2019). Pools of reactivity can be related to the temperature required to catalyse them into reaction with oxygen in a short (<4 hours) period of time, with labile carbon reacting at lower temperatures than recalcitrant (Liu *et al.*, 2020).

The carbon cycle is heavily modified and exploited by humans. In particular, agriculture disrupts natural nutrient cycles and creates an extractive system, relying on artificial inputs and management techniques which are short-lived and unsustainable (Johnson, 2016). A natural system has a balance of photosynthetic input against the output in the form of losses from heterotrophic decomposition, while an agricultural system reduces photosynthetic inputs and increases losses from decomposition, redirecting the produce and associated nutrients away from the land to be concentrated elsewhere (Janzen, 2004). The more labile pools of carbon are particularly vulnerable to this land use conversion, as they have shorter residence times, and detecting the flux in labile pools can act as an indicator of overall environmental condition (Sahoo et al., 2019). However, changes in land use, particularly towards intensive agriculture (linked to deforestation and soil erosion) have also created a greater than natural flux between the atmosphere and the pools of carbon previously assumed as recalcitrant (Bhattacharya et al., 2016; Janzen, 2004).

Agricultural management causes carbon to be lost from soils faster than it is replaced, which contributes to the global warming effect. Around 8% total carbon stocks stored in the world's soil is estimated to have been lost over the total period of human agriculture (Sanderman et al., 2017), accounting for 10-20% the total carbon emitted into the atmosphere since the industrial revolution (Dunne, 2017). UK soil contains about 10 billion tonnes of carbon, about 40-60% of what was originally contained in the soil before the onset of agriculture (Environment Agency, 2019). Because around 70% the land area of England is under agricultural management (Environment Agency, 2019), this is a significant factor to be considered in our future land management strategies and emissions targets. At a cost of £177 million a year in direct loss of productivity and indirect costs such as clean-up and water purification, three main processes cause the rate of soil erosion to exceed the rate of formation across the UK as a whole: water erosion, wind erosion, and removal during harvest (Environment Agency, 2019). Agricultural intensification increases soil loss as hedgerows are removed, field size increases, and soil is made more susceptible to erosion from the processes named above (Environment Agency, 2019), in addition to disrupting soil biodiversity and the biological processes of soil, pedon and biofilm formation which increase soil resilience (De Moraes Sá et al., 2014; Girvan et al., 2005).

This is a global issue (Bronick and Lal, 2005; Muhammed *et al.*, 2018). The clearing of forest is driven mostly by agriculture, which does not return organic material to the soil on any scale which would contribute to soil health (Muhammed et al., 2018). Forest clearing amounted to 230 000 kha from 2000–2012 (Hansen et al., 2013). With the advent of industrial farming and large-scale inputs, organic inputs to boost soil organic matter (SOM) and soil organic carbon (SOC) have fallen (Muhammed et al., 2018). The US corn belt and western Europe have experienced the highest global levels of soil carbon loss, because this is where intense crop production has been practiced for the longest periods (Sanderman et al., 2017). A reduced SOC availability in the soil affects the amount of C plants can assimilate and direct into growth areas including roots; with roots as an important natural stabiliser and aerating factor in soil, a lack of a strong rooting system exacerbates the degradation of soil quality, accelerates soil erosion and reduces net primary productivity (Rattan Lal, 2010a).

Alongside the loss of the physical soil resource and decreasing soil health, food production is simultaneously facing challenges in the form of climate change impacts. In many food-producing areas, climate change is forecast to bring increased coastal erosion, drought or desertification, changes in weather patterns, greater intensity and frequency of natural disasters, and increased land degradation (Willis, 2019). Land degradation from the effects of climate change – storms, drought, flooding – and the consequent loss of carbon from soils create a circular feedback loop as the carbon fluxes into the atmosphere as the greenhouse gas (GHG) CO_2 (Bhattacharya et al., 2016). The predicted temperature increase of around $3^{o}C$ by the end of the century is likely to produce a SOC decrease of 11-16%, which equates to a decline in SOC stocks of 3-8% even if SOM additions were to be increased by 20% over current inputs (Wiesmeier et al., 2016). In addition to land degradation, increasing CO_2 concentration in the atmosphere is expected to both stimulate plant growth and affect microbial decomposition, and thereby increasing carbon turnover in the soil and limiting the capacity for carbon accumulation (Van Groenigen et al., 2014). Globally, under the current climate, SOC is negatively related to average annual temperatures (Lin and Zhang, 2012). The threats to food production from two directions – the degradation of soil resources and the effect of the changing atmosphere – are intricately linked by carbon.

1.2 Carbon and agricultural exploitation

Land degradation and agriculture are linked by the soil carbon loss by land use change (Roser and Ritchie, 2019). Despite the threats facing soil health, agricultural subsidies are still directed towards policies which do not encourage long-term sustainability; in the US, agricultural subsidies amount to $20 billion, and in the EU the subsidies of the Common Agricultural Policy (CAP) account for 43% the total EU budget at $58 billion (Henderson et al., 2015). Currently, the objectives of the CAP are focused on increasing agricultural productivity and technical progress, a fair standard of living for farmers, and the stabilisation of markets, supplies and prices (The Institute for Government, 2020). Subsidies in the USA are aimed at preventing farm size contraction, insuring crop revenue, subsidising crop production, and are currently expanding into energy and environmental regulation (Rausser & Zilberman, 2016).

This existing system of subsidisation in the agricultural industry does not address current critical needs in terms of sustainable provision of food and fibre for a growing population in a world facing climate and biodiversity threats. Soil erosion and degradation costs between $100 million to $44 billion a year in the United States when both direct (e.g. loss of productivity, cost of fertiliser) and indirect (e.g. the financial cost of mitigating the GHGs from lost soil, rehabilitating land, and extra water processing) are taken into account (Henderson et al., 2015). In the US, there is an estimated 60 years of harvests remaining in the soil (Henderson et al., 2015), but US agricultural policy encourages processes which contribute to soil degradation including intensification and monoculture, corn production which requires intensive processing, and heavy pesticide and fertiliser use (Angelo, 2009). In Europe and in the UK, the Common Agricultural Policy encourages some practices which directly contribute to soil erosion and degradation, for example diffuse contamination from agricultural intensification, merging multiple smaller fields into larger units which remove hedgerows and tree cover, and the over-application of fertilisers leading to leaching (European Environment Agency, 2016).

This means the true cost of the food we buy is not reflected in consumer prices, but in costs to society, through pollution, emissions offsetting, water and soil degradation, and waste (Henderson et al., 2015). The production of economically cheap but environmentally costly food sets taxpayers back by approximately $4.7 trillion per year in terms of subsidies, remediation costs, waste disposal, water management, loss of productivity in degraded land, health impacts of diet and agricultural practices, and mitigating effects of GHG release from soil and farming (Henderson et al., 2015). Using large-scale land management, including changing subsidisation, we can help to tackle the root issue of soil degradation and loss, and retain the carbon in the soil; therefore, the return on investment in the long term will outweigh the short-term difficulties around the current systems and subsidies. The integration of the emerging forms of management discussed within this thesis into subsidy systems could contribute to long-term soil sustainability. Therefore, robust assessment is required for streamlining our current systems to meet our needs in a sustainable way.

While the soil C is currently being lost into the increasingly saturated atmosphere, these issues are compounded by increased demands on productive land, namely, population growth combined with changing diets. The resource consumption required by food production is vast: the value of ecosystem services provided to us by the environment, including directly producing food and indirect requirements and resources to do so, are estimated at $135 trillion a year (Costanza *et al.*, 2014). Currently, due to manipulation of the carbon cycle within our agricultural processes, we produce more food than ever before, co-opting up to 55% net primary productivity of the earth (Rojstaczer, Sterling and Moore, 2001); however since the Sustainable Development Goals were introduced in 2015, global hunger has actually increased (FAO, 2019), with 821 million people undernourished in 2017, up from 784 million in 2015 (Willis, 2019). As the population is set to reach 9.6 billion people by 2050, the soil will be under more pressure than ever, providing the basis of all the ecosystem services we rely on (E. Milne et al., 2015).

SOM, and specifically SOC, content of soils is critical to the provision of ecosystem services; multiple benefits have been identified when soil carbon is increased including nutrient cycling, water regulation, habitat provision, and climate regulation through carbon sequestration (Milne *et al.*, 2015). As a key player in global land management, agriculture must be integrated in measures to prevent and possibly even reverse SOC loss. The level of SOC is a key way of evaluating the sustainability of the current soil management and agricultural practice on a site, as it suggests soil structure and fertility (Chen *et al.*, 2018). Soil structure – the degree of stability of aggregates – is mediated by SOC, which bonds primary soil particles together (Bronick and Lal, 2005). The soil C is held in compounds, such as the glucose, glycine and citric acid which are common in plant root exudates and incorporated and transformed in the microbiome of the soil (Eilers *et al.*, 2010). Compounds driving soil structure are released by the soil biota, breaking down primary carbonates and producing organic cations which increase the precipitation of secondary carbonates, while the roots of plants and fungal hyphae physically adhere particles together (Bronick and Lal, 2005). This has the positive feedback effect of increasing soil C sequestration capacity (Bronick and Lal, 2005), and increasing water and nutrient retention (Jiao, Whalen and Hendershot, 2006; Sun and Lu, 2014; Bucka *et al.*, 2019). SOM will be crucial in addressing food security, adaptation to climate change, and mitigation of greenhouse gases (GHGs) and the UN is encouraging a voluntary action plan, the '4 per 1000 Initiative: Soils for Food Security and Climate' to bring an annual increase of 0.4% in global SOC stocks, focusing on agricultural land (Soussana *et al.*, 2019).

Plant diversity in grasslands increases biomass production and SOC storage (Cardinale et al., 2007; Chen et al., 2018; Craven et al., 2016; Hector et al., 1999; Mellado-Vázquez et al., 2016). Increased plant diversity increases the availability of recently photosynthesised carbon in the rhizosphere to the microbial community (Mellado-Vázquez et al., 2016). Agricultural practices affect soil communities: short term changes such as land use and loss of habitat affect soil mesofauna more than microfauna, whereas longer term changes such as loss of organic matter (ultimately a source of carbon for the soil) has a greater effect on microfauna (Postma-Blaauw et al., 2010). Mesofauna suffer greater falls in abundance, diversity and functional group diversity than microfauna, but all groups see a reduction when soil is under agricultural management as opposed to a natural system (Postma-Blaauw et al., 2010), and the mesofauna is ultimately responsible for the first step of organic matter turnover, from which the microbial community derive their energy sources (Filser et al., 2016). Nitrogen enrichment, ubiquitous in agriculture, increases productivity and SOC storage, but decreases species richness (Chen et al., 2018).

1.3 Soil carbon storage mechanisms

The physical, chemical and biological processes of SOC stabilisation are interlinked. The carbon in soil comes from biota - plants and organic matter, from excreted, exuded, or dead tissue (De Neergaard and Gorissen, 2004). Roots and hyphae also bond the soil particles to create larger aggregates (Rillig & Mummey, 2006), create soil pores of different sizes, and influence the overall soil structure

(Bronick & Lal, 2005). So does the presence of soil 'engineer' macroorganisms such as termites, ants and worms (Bray et al., 2020; Lavelle, 2002). The biological action of the microbiota not only breaks down tissues into chemical compounds for integration into the soil, but also develop the soil microbial crust which is a cohesive layer aiding soil stability and robustness to physical changes such as drought (Garcia-Pichel et al., 2013). Physically, the structure of the grain is influenced by the compounds and cations which 'bridge' between primary soil particles and influence aggregation (Bronick & Lal, 2005). Carbon is a key element in the bridging compounds, and thereby in building up a soil structure, and the effectiveness of carbon integration into the soil depends on the C breakdown by the microbial community (a biological process) and the presence of cations (a chemical process) which promote dissolution of primary carbonates and formation of secondary carbonates such as (hydr)oxides and phosphates which enhance aggregation (Bronick & Lal, 2005). Metallic-based cations (for example iron, aluminium or calcium ions) also enhance precipitation of bonding compounds (Bronick & Lal, 2005). The presence of water catalyses the biological processes of root and fungal hyphae growth for ped stabilisation (Reubens et al., 2007; Rillig & Mummey, 2006), which then increase physical soil resilience to drought. Water influences plant and microbial community composition, biomass, and richness, and the associated exudates and C compounds and concentration (Michalet et al., 2018). Water is a vehicle for chemical transport via the action of raindrops, flow, diffusion, and collection in pores (Gao et al., 2004). Water is also a physical actor in the form of freezing and melting which expands cracks and pores (Gharedaghloo et al., 2020). The interrelated complexity of soil biota and the associated chemical and physical processes and properties are all deeply reliant on the presence of carbon, while the cycle of water influences the speed and integration of carbon back into the system.

The particle scale of geological parent material influences soil texture, which is a factor in SOC storage potential. For instance, increased clay content increases storage capacity, but SOC stabilisation is also dependent on mineralogy (Bhattacharya et al., 2016; Kögel-Knabner et al., 2008; O'Rourke et al., 2015) and respiration from SOM decomposition by the microbial community (Xu & Shang, 2016). In terms of mineralogy, the base material influences aggregate stability, seal formation, runoff and soil loss (Lado and Ben-Hur, 2004). The parent material, the bedrock, also determines likely grain size in the soil matrix and influences soil pH (Eze, Palmer and Chapman, 2018; Bucka *et al.*, 2019). Grain size and mineralogy are chemically linked: up to 65% of the organic C in mineral topsoils occurs in young, particulate matter that is loosely or unconnected with soil minerals and can be rapidly reacted (Kaiser *et al.*, 2002). Soil pH also affects and is affected by SOC and SOM, as the negative surface charge on clay particles increases with pH, which increases particle repulsion, and almost all soils have some element of clay (Bronick and Lal, 2005). SOC and SOM are thereby indirectly affected by the metal ion solubility and clay dispersion depending on pH, while microbes which assimilate soil carbon may find some pH levels inhospitable and the community activity or composition will also fluctuate with pH (Bronick and Lal, 2005). While SOC protection processes are chemical, SOC contributes to soil physical behaviour; turnover rates for carbon depend on the time molecules are sorbed on to silt and clay particles to form organo-mineral complexes, and where the molecules are stabilised (Kögel-Knabner et al., 2008). In addition to texture and chemistry, the 22-year study by Kahlon et al. (2013) showed that soil structure, influenced by management, was the key indicator of carbon sequestration capacity. Dividing the impacts by scale (particle, aggregate, pedon, landscape, biome and biosphere scale effects) is a very useful way of translating effective managements for SOC into policy for soil security (O'Rourke et al., 2015).

The systems by which carbon is stored are crucial to making informed management decisions, but they are not well understood, with two main approaches to quantifying (and particularly to modelling) soil C. The first, carbon pools, is very well established in the literature and still heavily relied upon (Parton *et al.*, 1994; Hartmann and Niklaus, 2012). The second, steady state, is an emerging school. This is because although the theory is more than twenty years old (Cook et al., 1998), modelling has only recently reached the capacity to incorporate the required processes and timescales (Fang *et al*., 2014).

The carbon 'pools' theory is easier to visualise, assess and model. Dignac et al. (2017) describes SOM as non-homogenous, with conceptual 'pools' mineralised into the soil and persisting at different rates and times; however, the study describes links between SOM and chemical fractions as 'questioned'. The lability or recalcitrance of the C defines the pool – how reactive the C is, and how accessible to the wider environment (Cotrufo et al., 2015). Bronick & Lal (2005) describe the system as the more labile pools being utilised first to fuel microbial activity, the supply decreases and the soil macroaggregate which was initially formed around particulate organic matter (POM) loses stability. This eventually releases the more recalcitrant carbon which formed in microaggregates within the macroaggregate particle (Bronick & Lal, 2005). Timescales for the formation and depths of these fractions of reactivity vary greatly with soil management (or lack of), nutrient deposition, seasonality, temperature, and the other factors involved in carbon mineralisation (Currey et al., 2010; Stuble et al., 2019). More recalcitrant C also tends to be deeper in the soil where turnover time is extended away from reacting with oxygen, where it is transported by a healthy macroinvertebrate community e.g. in worm casts, and where subsoil microorganisms produce stable compounds (Lorenz & Lal, 2005).

Steady state theory describes soil reaching a 'steady state' when SOC remains stable under continuous soil management practices and constant C input levels, which balance the overall C against SOC stocks and SOC mineralisation (Nicoloso et al., 2016). The steady state theory still assumes carbon is in pools, which vary in turnover rate with recalcitrance (Cole *et al.*, 1993). However, the assumption of constant behaviour does not necessarily indicate that the outcome will hold in steady state, and is heavily dependent on the data available (Andrén et al., 2004). Steady state theory is an important factor in determining the ultimate capacity of a soil at landscape scale to advise management, to ensure realistic projection of capacity and efficient management (Nicoloso et al., 2016). Steady state assumes carbon is in pools, but pools do not necessarily rely on steady state.

In terms of practical application in agricultural land management, Pausch & Kuzyakov (2018) rely on the segregation of carbon into pools to illustrate the input of C by rhizodeposition, which interacts with management depending on whether the cover is crop or grassland, and the timescale of establishment. This review relied on 44 studies which all used the C pools framework across diverse ecosystems and treatments, including cropland and grassland (Pausch & Kuzyakov, 2018). The Hatton et al. (2015) study also firmly adhered to the pool theory, showing at least two pools with different turnover times coinciding with different SOM fractions.

However, Pausch et al. (2016) demonstrate that C pool size and labelled ^{13}C do not match closely. Recent research has shown that lignin does decompose in all stages of decomposition (Talbot & Treseder, 2012), rather than accumulating to a specific recalcitrant pool (Cotrufo et al., 2015). The percentage of C remaining in a pool does not reflect the rate of flux through that pool, which can be much larger, and therefore processes which drive C assimilation in the soil cannot be evaluated on pools alone and the flux should be incorporated (Pausch & Kuzyakov, 2018).

A major issue with examining the steady state theory is that models have to 'spin up' for hundreds of thousands of years to reach a dynamic steady state, which is expensive and difficult (Fang et al., 2014). Steady state modelling means variables are stable over longer timescales but allow variability over shorter timescales (Fang et al., 2014). Nicoloso et al. (2016) evaluated three mathematical models based on a 17-year experiment in Kansas, US, assessing soil tillage and no-tillage systems, which indicated that no-till soil systems can act as a finite C sink until they stabilise at steady-state levels at between 20-50 years of establishment, where C reaches equilibrium. The models vary with their estimations of SOC dynamics, because of the assumption that increasing C inputs always promote a linear increase in steady state SOC, and other studies where SOC levels are at steady state or saturation are scarce (Nicoloso et al., 2016).

1.4 National and international commitments to soil health and soil carbon

Acknowledging the strands of C-related risks to our future capacity for resource provision in a regulated climate, and integrating them into soil management, is a crucial step towards a sustainable future. Here in the UK, we are beginning to see soil and its C capacity incorporated into possible future mitigation strategies, as well as acknowledging the risks presented by current management (DEFRA, 2022a).

Agricultural soils have been the target of improvements over the past decade. The ‘Safeguarding our Soils’ strategy for England was published in 2009 with the aim of managing all English soils sustainably and reducing all forms of degradation (DEFRA, 2009). Unfortunately, none of the goals were binding and most were missed, including agricultural soils being better managed and threats addressed, which has demonstrably proved not to be the case since the publishing of the strategy in 2009, with 2 million ha of soil still at risk of erosion, costing £1.2 billion each year (Environment Agency, 2021). The goal of soils in urban areas being valued during development has not materialised in planning permissions or development considerations, as there is no governmental guidance section in the Planning and Building section of the governmental planning policy portal, and soil is not mentioned in the Green Belt, Climate Change, or Effective Use of Land guidance, although it makes a brief appearance in the Natural Environment section (DLUHC and MHCLG, 2016). The latest code for sustainable use of soils on construction sites dates from 2011 (DEFRA, 2011). Pollution of soils has not been prevented, with a total of 300,000 ha of land in the UK now contaminated by various pollutants from chemicals to microplastics (Environment Agency, 2021). Between 2000-2013, only 1,000 of the 11,000 sites of contamination received any form of remediation (Environment Agency, 2016). Organic matter decline was another aspect the Strategy aimed to address, the effects of which are still proliferating by the drainage of peatlands and ploughing up pasture for arable crops or even temporary grasslands (Environment Agency, 2019). The only aspect of soil heath which may have been addressed is its role in climate change as a soil sink, rather than soil carbon for soil’s sake (Payne and Jessop, 2018; Poppe, van Duinen and de Koeijer, 2021). This has become better known as a piece of the puzzle in policy-making around climate (Minasny *et al.*, 2017; Soussana *et al.*, 2019; Trenbirth and Dutton, 2019), however, the role of soil carbon has been focused to a great extent on peat (Davies *et al.*, 2016; IUCN UK, 2018, 2022; DEFRA, 2021a). Peat is a particularly high-C soil vulnerable to environmental pressures, which has become a net source of C after previously acting as a net sink (Wang *et al.*, 2021). 1,685,562 ha peatland is used to support some form of agriculture in the UK, so some of this policy is still relevant to agricultural land, but not to the greater proportion of land in England which is not peat – some 88% (Trenbirth and Dutton, 2019).

In 2016, a comprehensive report into soil degradation across the EU was published, which accepted that current policies only addressed soil as a by-product of other anti-pollution regulations rather than treating it as a valuable resource in its own right with accordant protections (European Environment Agency, 2016). In the UK, the government established a 25 Year Environment Plan in 2018 which requires that British soils be managed sustainably by 2030, and steps must be taken to restore that which has been lost (UK Government and DEFRA, 2018). These include the Environmental Net Gain principle for development, which is now commonly implemented; changing the incentives around land management with environmental land management schemes (ELMS) (beginning to established on a wide scale between now, 2022, and 2024) and phasing out the Basic Payment Scheme for landowners, to be ended in Wales in 2021 and across the rest of the UK by 2028. Ending the use of peat in horticulture is currently scheduled for 2030. Additionally, restoring woodlands and making greater use of natural flood management solutions are also being incentivised (Forestry Commission, 2022). These are positive steps towards not only setting goals, but establishing networks and implementing real strategies to actually produce change. These solutions address wider issues which have substantial impact on soils, but the report also addressed soil-specific goals such as £200,000 of DEFRA funding for developing metrics for soil health and rolling them out nationally, thereby increasing monitoring to address the knowledge gaps in policy effectiveness (UK Government and DEFRA, 2018).

In 2019, a national audit was carried out by the Environment Agency named The State Of The Environment, and soil was named a key degraded environmental factor (Environment Agency, 2019). New Farming Rules for Water were initiated to aid farmers in a catchment-based approach to reduce field erosion, and ELMS, brought in to phase out Entry Level and Higher Level Stewardship schemes, has created an opportunity to encourage farmers to protect and regenerate soils through payments encouraging sustainable farming practices and meeting local biodiversity targets (Environment Agency, 2019).

In combination with the practical benefits, soil carbon management can also help the UK to meet international voluntary and legal commitments. The UK is committed to reducing emissions by 57% by 2032 (Department for Business, Energy and Industrial Strategy, 2018), and carbon emissions contributions from soil are significant (Ball, 2013; Dunne, 2017; Farrar & Williams, 1991). Meanwhile, improving soil health and restoring carbon to the soil will help fulfil several Sustainable Development Goals (United Nations, 2020): zero hunger (goal 2) and good health (goal 3), which include nutrient deficiency; the clean water and sanitation (goal 6) correcting some of the pollution which originates from farmland run-off, including siltation; goal 11 for sustainable communities; goal 12, encompassing responsible consumption and production; the climate action improvement aims (goal 13); and the life on land approach (goal 15) which includes species in areas which are being limited by land use change (UN, 2015.). The ‘4 in 1000’ soil carbon storage initiative to establish an annual growth rate of 0.4% in the soil carbon stocks under the Lima-Paris Action Plan (“4p1000” CGIAR System Organisation, 2018; Dignac et al., 2017; Minasny et al., 2017).

1.5 Soil carbon improvement in other land uses, and potential application to agriculture

1.5.1 Soil carbon and forestry

Some parallels can be drawn with tree planting and urban forestry which takes place on land degraded by similar processes to agriculture, such as compaction, resource extraction and/or contamination. Carbon storage and sequestration in the soil have been identified as possible benefits of afforestation (Ward et al., 2021), the practice of transforming degraded or abandoned land into forest or woodland (Santos et al., 2019). As a low-cost nature-based mitigation strategy with multiple possible climate, environmental and social benefits such as carbon storage, biodiversity gains and mental health applications, afforestation even on previously unforested land and urban areas has seen a rise in popularity. Healthy soils help retain both storm water and any nutrients which may otherwise be lost from run-off, which would help support the growth and establishment of planted trees alongside the human social benefits of reduced urban stressors (Ward et al., 2021).

Urban afforestation can contribute to SOC pools and reduce soil compaction to aid water infiltration and the added benefits that are brought with water such as microbial activity and changes in pH, aiding long term urban soil recovery (Oldfield et al., 2014). However, afforestation on degraded urban land to the benefit of the soil first relies on the soil retaining any capacity to support the growth of the plantation, and site treatments such as compost amendment and interplanting with shrubs have been examined (Downey et al., 2021; Ward, et al., 2021).

Forestry approaches tend to be on a much longer time scale than any potential agricultural land management, especially the schemes currently in practice in the UK. For instance, Jiang et al. (2013) took forestry data from a 70-year span. 5-6 years may also be a common study period but it is accepted that during this time, no trees are going to reach maturity and their growth patterns are going to have continual effects on the soil for much longer (Ward, Doroski, Felson, Hallett, Oldfield, Kuebbing, Bradford, et al., 2021). However, soil depth may be considered down to 1 m in some studies due to the root depth of trees (Downey et al., 2021) which is useful for understanding the impacts of trees on a soil horizon and carbon store less disturbed by human activity.

Rehabilitating contaminated soils

Afforestation has been shown to increase SOC stocks on former mines, depending on soil factors such as pH, tree species or functional group (Mayer et al., 2020) as these soils are classed as in ‘early stage of development’ (Hüblová & Frouz, 2021). Likewise, it has been shown to provide carbon benefit on former landfills (Ashwood et al., 2018). However, as we have seen in agriculture, some of the management strategies have both positive and negative outcomes: a popular method of increasing both water storage and rooting potential for newly planted trees is tillage, but we know this decreases the overall SOC content initially (Dignac et al., 2017; López-Fando et al., 2007; Ward, Doroski, Felson, Hallett, Oldfield, Kuebbing, & Bradford, 2021). While the C loss from soil is likely to be more than compensated for in atmospheric terms within the body of the established trees, whether its full replacement in the soil will be achieved merely by tree plantation is debated, especially on peat (Payne & Jessop, 2018). Forestry for rehabilitating the soil to increased carbon levels can also take far longer period than most land managers can contemplate – up to 100 years (Mayer et al., 2020). It is also unclear whether there are particularly effective tree species for remediating soil into a condition where it is suitable for other uses such as amenity land or agroforestry (Heckenroth et al., 2016; Roy et al., 2007).

Additives and inputs

Additives in the form of compost, green waste or biosolids can have a positive impact on soil condition depending on the baseline state (Ashwood et al., 2018; Smith et al., 2005), and therefore on tree establishment and growth rates (Bulmer et al., 2007; Somerville et al., 2018). The Ward et al. (2021) study on interplanting, compost addition and species composition within plots in New York City found significant increases in the soil C, N, microbial biomass and water holding capacity were produced over just six years across all treatments. The largest differences and strongest variable interactions were produced by compost addition: mean C increased by 35% from 2009 to 2016 in compost-amended plots, with no change in the control plots (Ward et al., 2021). While N saw a 21% increase even in control plots over the same period, a 92% increase was observed in compost-amended plots. However, no significant main effects of any of the three amendments was observed over the six years across every single plot, and there was a confounding effect: while the C stocks of compost-amended plots increased compared to the baseline (up 16.7%), the control plots with no amendments had significantly lower stocks compared to the baseline (down 10.4% over the same 2009-2016 period) (Ward et al., 2021). While soil C gains were also highest in the first year post-planting, the increases diminished year on year thereafter with a plateau after the third year, and microbial biomass plateaued after the first year (Ward et al., 2021). Despite the reduction in gains over time, this is a positive indication of the improvements that can be made in soil microbial biomass, N and C, which are all related to each other and to important soil functions such as water holding capacity and vegetation support (Oldfield et al., 2014). It is likely that over a much greater timescale, if the natural processes are allowed to progress, the soil may return to a more established and stabilised state of nutrient cycling under established forest (Warner et al., 2022). A mature and undisturbed soil allowed to support a developing woodland is likely to remain in a positive sink for carbon over the course of natural succession (Warner et al., 2022), and thereby even if gains are marginal, the prevention of loss after the initial increase in SOC post-planting would be of benefit.

1.5.2 Soil carbon in arid and semi-arid soils

Semi-arid and arid soils have also been an area of focus for soil carbon as a catalyst for soil health improvements. Dry soils do support agriculture across the world, be it animals reared on semi-arid grasslands, or irrigation for arable production. A large area of China is in arid and semi-arid climates, with soil erosion and desertification a significant problem which is increasing in terms of land area at about 2% a year in China; the area of degraded grassland increased by 95% between 1989-1998 (Lal, 2002). Desertification leads to emission of CO_2 by soil loss from erosion and the loss of the biological community, and thereby processes by which C is stored in in biomass and maintained in compounds including plant roots, microbial exudates and biofilms (Lal, 2002). However, there are several ways to help prevent desertification and retaining soil carbon is a key component in many of the practices currently being adopted such as conservation tillage, organic matter inputs, precision farming, controlled grazing, optimal irrigation, nutrient retention, and soil structure optimisation (Rattan Lal, 2004). It is important to understand what has previously worked in arid and semi-arid soils even when aiming to better understand more temperate and wetter soils, as dry soil is often on the front line for facing climate-driven issues, including future temperature increases and drought likelihood, with soil condition and management which may become more common in temperate areas under climate change, requiring changes to soil management.

Irrigation

Irrigation is also key in these areas as water is necessary for plant growth. Irrigation increases crop production and survival (Rattan Lal, 2004; Denef *et al.*, 2008). However, irrigation has been indicated to i) increase SOC stocks by increasing productivity and residue inputs (Follett, 2001), ii) decrease SOC storage by stimulating microbial activity and OM decomposition (Churchman and Tate, 1986), and iii) broadly make little difference as inorganic carbon, root activity or pH (Denef *et al.*, 2008) are greater drivers of soil C cycling. However, increased soil water content increases dissolution of CO_2 in soil air spaces into biocarbonate and carbonic acid (Denef *et al.*, 2008), thereby increasing the residue and litter input into the soil by increasing plant growth (Entry, Sojka and Shewmaker, 2002) and stimulating some species of cyanobacteria to form an increased protective crust (Román *et al.*, 2021). Entry et al. (2002) found that converting native sagebrush to irrigated ploughed crops would lose 0.15 g C m^{-2} from the soil annually, but converting it to irrigated pasture would sequester 3.56 g Cm^{-2} y^{-1}; similarly, converting irrigated ploughed crop to irrigated pasture would sequester 3.71 g C m^{-2} y^{-1}. Optimal irrigation in the North China Plain increased SOC storage down to 1 m depths by 0.88-1.86 t ha^{-1} y^{-1} compared to conventional agriculture (Zhao *et al.*, 2013).

It should be noted that while irrigation may encourage soil carbon storage in some circumstances, the environmental costs of irrigation can be high. Water extraction can limit supplies in other areas (Yang, Zhang and Zehnder, 2003). Additionally, increasing water can increase nitrogen export in the form of NO_2 and NO_3, GHGs with high warming potential (Isidoro et al., 2006), which is further explored in Chapter 6.

Tillage system

Changing the tillage system is becoming more common as it is accepted that reducing or eliminating tillage can increase SOM in the top 5 cm of agricultural soils and improve soil porosity and water filtration (Brown et al., 2021; García-Marco et al., 2016; H. Wang et al., 2020), and may reduce N_2O emissions especially in drier climates (van Kessel et al., 2013); however, N_2O emissions may increase under minimum or no tillage regimes due to the application of additional fertiliser (Powlson et al., 2012), especially in more temperate or humid conditions (van Kessel et al., 2013). Minimum or no tillage in semi-arid areas with a monsoon climate decreases CO_2 flux to the atmosphere by 14.5% compared to plough tillage, and after 11 years increased SOC storage in the dryland soil by 5.85 mg ha^{-1} compared to soils that were ploughed (H. Wang et al., 2020). It is also an effective measure towards minimising energy and machinery use, controlling physical erosion, and can improve water and fertiliser efficiency (Triplett & Dick, 2008). No tillage has been adopted in areas receiving as little as 250 mm of precipitation per year (Rolf Derpsch et al., 2010).

Minimum or no till systems are fairly well understood in their impacts on the soil, practiced in every climate and soil type that supports a form of agriculture (Rolf Derpsch et al., 2010) and with around six decades of research and data to rely on (R. Derpsch et al., 2014). However, no till is obviously not a cure-all otherwise tillage wouldn't persist, and tillage does bring benefits including reducing weed competition and surface roughness, promoting water filtration, quicker warming of the soil in spring, and improving organic matter mineralisation (Triplett & Dick, 2008).

The definition of 'no till' is elastic, used for areas which have had no tillage for any length of time, no matter how short; for any period of crop cover or lack of; for any additive or input level; and across both monocultures and polycultures (R. Derpsch et al., 2014). Because of this, no till or min till systems can show variable effects on soil emissions (van Kessel et al., 2013). Many studies also take advantage of this long history of no till data and study the impacts over time, up to fifty years (Jha et al., 2022) but this means it may be unhelpful to generalise the effect of no tillage over very short timescales, such as the lifespans of temporary leys (generally 1-5 years) which are the subject of this thesis. In the experiments of later chapters, no tillage will be a factor in management, but the literature indicates that it will not be a deciding factor in so short a timeframe.

Inputs: green manure, biochar and organic mulch

The application of manure, green manure, biochar and mulches has also been used to positive effect on SOC in arid and semi-arid areas, depending on the type of biochar, initial soil status, and whether it is used in conjunction with other additives. The application of both goat manure for SOC input and charcoal to stabilise the SOC and enhance nutrient and water retention has been effective in Northern Oman, with organic treatments increasing SOC by up to 21% and N up to 48%, while inorganic inputs reduced SOC by 25.5% and N by 20%; charcoal amendment increased SOC by 10.6% when fed to goats or by 21.3% when charcoal was mixed with manure and then applied (Ingold et al., 2015). Biochar absorbs nutrients, which improves soil fertility and thereby promotes plant growth and crop yield, but also reduces contamination and run-off (Ding et al., 2016). Green manuring in the semi-arid Indian tropics significantly decreased soil bulk density and increased SOC, carbon sequestration potential rate, water infiltration, and available NPK over a period of five years; the crop yield increased by 42.3% under green manure application (Premi et al., 2013). In Rawapindi, Pakistan, the application of biochar with N fertiliser was studied on an arid soil for two years by Irfan et al. (2019) and found to increase microbial biomass carbon by 18% and nitrogen content by 63% across both study years, indicating a significant improvement in soil health, nutrient status, and microbial function.

This is useful to know the positive effect of organic inputs on soil C even on timescales of <5 years, which is relevant to the time cycle of temporary agricultural leys focused on in the later chapters of this thesis. However, the literature is lacking when it comes to the depth effect of these inputs and the longevity of the impacts in the soil – potentially the impacts only reach the top 30 cm of soil and are quickly synthesised – as well as failing to positively impact other soil health indicators such as nutrient content (Blanco-Canqui et al., 2020). It is possible that the effects of biochar only persist as a positive legacy, when the C from it is stabilised over periods as long as centuries or millennia, including at depths lower than agricultural activity can generally disrupt (Lorenz & Lal, 2014).

Inorganic mulch

The application of mulch, a layer of any material on the soil surface (commonly bark in temperate or humid areas, but more commonly gravel or stone in arid areas) is usually targeted at moisture conservation, but as above, moisture is linked to SOC and soil fertility. Gravel mulching can help prevent soil erosion and retain moisture (Du et al., 2022; Wang et al., 2011). Applying gravel mulch in the arid Tibetan Plateau provides a more hospitable environment for SOC and N as moisture is retained and soil protected from wind erosion; sites with gravel had SOC stocks of 49.6 mg ha^{-1} and total N of 2.8 mg ha^{-1} compared to gravel-free sites which contained 29.5 mg SOC ha^{-1} and 1.4 mg N ha^{-1} (Wang et al., 2011).

Applying inorganic fertiliser and then the gravel could, however, decrease SOC due to reductions in the macroaggregate content, associated degradation of other physical and chemical soil parameters, and the pressures of continuous cropping (Du et al., 2022). Therefore replacing chemical fertiliser with organic matter and subsequently gravel mulching the area has proved to be a more effective way of utilising gravel mulch as soil protection to maintain or increase SOC (Du et al., 2022).

Many studies aim for the longest experiments possible, understandably for the reliability of analysis (Kahlon et al., 2013; F. Wang et al., 2022), whereas this thesis aims to investigate soil managements which can impact soil C in the long term by short-term or cyclical intervention. It also seems that mulching for vegetable and soil health over a short period relevant to ley cycles (~two years) suggests that the impact of mulching is inconsequential compared to compost application and biodegradable mulches are unlikely to have an effect on the soil due to the time it takes them to degrade (Reid, 2019), although there is little applicable research on timescales for mulching effects in the short term.

Cover crops

Cover crops, the growth of a crop which is not necessarily profitable but which protects the soil, have also been utilised in arid and semi-arid areas. Cover crops physically cover the soil and protect it from wind erosion; absorb water run-off; increase water filtration into the soil by increasing soil porosity (Ruiz-Colmenero et al., 2013); and absorb nutrient run-off, helping to cycle it in the soil and improve productivity rather than allowing damaging wastage into watercourses (Snapp et al., 2005). Leguminous cover crops also have the benefit of fixing N for the rotational crops, as well as producing high quality residues (Snapp et al., 2005). Permanent cover crops (which also necessitate a no till regime, so the effects accrue in tandem) proved to increase SOC and nitrogen down to 5 cm depths in a northern Spanish vineyard of semi-arid climate (Peregrina et al., 2012). In a Mediterranean orange orchard, cover cropping between the fruit trees did not significantly improve SOC in total, but labile C in the top 10 cm of soil was significantly improved, with benefit to the fruit trees (de Oliveira et al., 2016). The concentration of SOC in sediment was greater in cover crops than under traditional management in another Spanish study, with a loss of 0.06 t SOC ha^{-1} y^{-1} under tillage compared to a loss of 0.02 t ha^{-1} y^{-1} under cover crops (Ruiz-Colmenero et al., 2013). In semi-arid southern India, a legume cover crop between sorghum crops and sunflower crops was analysed over 9 years and proved to increase mean SOC by 24% compared to leaving the soil fallow, or compared to a decline under long-term application of chemical fertiliser only (Venkateswarlu et al., 2007).

When cover crops are used in tandem with a minimum or no till regime, it can be difficult to differentiate the effects, and lower horizon C gain from cover crops is not well understood (Patra et al., 2022). Additionally, the impact on C of the length of time the cover crop is established is not well understood but it could take 155 years to reach carbon saturation using a cover crop (Poeplau & Don, 2015). Understanding short term impacts is one of the factors addressed by experiments used in this thesis to simulate temporary leys.

Perennial culture and agroforestry

Perennial culture and agroforestry tend to be used in tandem, like manure and biochar, or mulch and fertiliser, in arid and semi-arid soils. As perennials flower and fruit more than once, they require less cultivation and no digging, which is beneficial in limiting soil disturbance to avoid physical erosion and for tapping into ground water in areas of scarce supply. They are a key component of semi-arid agroforestry, where drought-tolerant trees or shrubs are grown in a matrix with crops or pasture. Competition for light and resources must be managed, but if successful, the complementary effects on soil fertility exceed any detractors (Sanchez, 1995). Agroforestry in semi-arid Kenya showed that native trees or trees from a similar climate and environment are important for the balance between cover and nutrient availability, as non-native species drop their leaves at times which do not allow them to take the full benefit of the short rain periods (misaligned phenophase); however, the indigenous trees retained some leaves and remained hydrated even in periods of drought, while some dropped leaves provide a residue to improve soil health (Broadhead et al., 2003). Kenyan farmers often utilise a fast-growing and deep-rooting indigenous tree, *Melia volkensii*, to maintain agroforestry with a variety of crops (Stewart & Blomley, 1994). When trees are dispersed within the crop at 10-15 m intervals and regularly pruned, *M. volkensii* provides good quality timber as well as providing shade to the adjacent crops, a key issue for crop health in arid and semi-arid systems (Stewart & Blomley, 1994). However, agroforestry can lead to significant issues: although temporal separation through the growing periods was maintained, a lack of spatial separation of tree and crop rooting activity led to competition with reduced maize yield and soil moisture, which outweighed the benefits of the provision of shade and soil aeration and increased porosity from the trees in a separate Kenyan study (Odhiambo et al., 2001). Agroforestry has long been an indigenous practice in the arid regions of India to reduce water losses when evapotranspiration exceeds precipitation, and to improve the physical condition and nutrient levels in the soil; trees tend to increase available organic matter and soluble C, reduce soil $CaCO_3$, and as a result, decrease the soil pH from an average of 8.2 to 8 or below, as trees tap into deeper soil resources (Shankarnarayan et al., 1987). This not only increases the availability of some nutrients and the hospitability of the soil to a greater range of crops, but also has the benefit of separating the resource pools of deeper-rooting trees from shallower-rooting crops to avoid competition (Shankarnarayan et al., 1987).

The processes by which woody perennials become established for horticulture in arid regions can be difficult to control or understand. Available information on transplanting and seeding can be fragmented and soil processes have not, historically, been well accounted for (Azam et al., 2012). The long-term implications of arid agroforestry which often utilises non-native species is also unknown (Ong et al., 2006). Depth impacts may also be neglected when soil C analyses focus on the O and A horizons rather than deep effects which may result from the influence of deep rooting trees (Yadav et al., 2011).

1.6 Agricultural efforts to restore carbon

There are now several recommended management practices evidenced by published studies which aim to increase SOC stock. Common approaches directed towards the purpose of carbon increase include mulch farming, conservation tillage, diverse cropping systems, cover crops, agroforestry, and integrated nutrient management (Lal, 2004). Theoretically, the potential to sequester carbon back into agricultural soils could be as high as 20-80 tons ha^{-1}, the ‘gap’ left by C emitted into the atmosphere through previous poor management practice (Lal, 2004). However, the attainable soil sink capacity is only 50-66% the potential capacity, because the resilience of the soil itself has been damaged, and the rate of sequestration of secondary carbonates is low (Lal, 2004). The actions required to sequester the carbon also come with a carbon cost: agricultural inputs containing C must be produced and distributed (including pesticides, fungicides, herbicides); pumping water for irrigation takes up to 150 kg C ha^{-1}; and ploughing, drilling and harvesting all require carbon in terms of vehicle emissions (Lal, 2004). These are not including secondary considerations such as distribution, and the process of losing C from the perturbation of soils through tilling and similar activities. Therefore, C lost from soils is unlikely to be completely replaced, or even the loss entirely halted. Certainly, in the next few decades, the solution must rather be based in preventing as much C loss as possible at the outset. Continuing to monitor and improve the solutions as they are researched and tested may bring emissions reductions on an ever more efficient scale, according to local soil types, biodiversity, agricultural products, and management practices.

1.6.1 Crop rotation and cover crops

Crop rotation is one of the oldest methods for ensuring soil health, in use as early as 6000 BCE and localised to complimentary regional crops and timescales, ranging from three fields rotated each year in medieval Britain to 36-year cycles in central Africa (R. E. Stewart, 2018). The approach depends on the assumption that different crops consume and exude resources in the soil at varying concentrations and rates, and some, especially legumes, leave a legacy effect which will benefit future crops (Paine & Harrison, 1993). Chosen rotations or tillage methods often have cultural, environmental, or economic restrictions, and are not generally targeted at soil C specifically (Rickman et al., 2002). The impact of rotations on soil C also depend not only on the crops and durations they are established, but the biotic and abiotic factors of the soil and landscape such as rainfall, the number and depth of soil layers, the residues added, and even temperature (Rickman et al., 2002).

Cover cropping is generally used in tandem with other established methods for returning SOM e.g. no till or organic matter inputs. Generally serving a dual purpose as either a protector of the soil, especially in arid or exposed areas, or a green manure, the cover crop is also usually used for grazing, silage, or a harvest of a winter crop (Paine & Harrison, 1993).

Benefits of crop rotation can be evidenced in harvests as well as detailed soil analysis: a meta-analysis of crop rotation showed that crop yields increased by up to 20% compared to monocultures in China (J. Zhao et al., 2020); a completely input-free study in Michigan, US, showed that corn yields increased over 100% in the most diverse treatment (three crops interspersed with three cover crops) compared to monoculture (R. G. Smith et al., 2008); A further incentive for soil health, which is both driven by and a driver of SOC accumulation, crop rotation provides benefits by reducing the build-up of soil borne plant pathogens, which can thrive in a multiple-season monoculture and increase disease transmission, by up to 50% (Larkin et al., 2012). This suggests legume-based and complex rotations must be adopted to enhance biomass production (Lal, 2013).

1.6.2 Land fallowing

Alongside crop rotation, land fallowing (leaving areas unplanted, and allowing natural encroachment) was the earliest human approach to restoring soil health and fertility; as farmers recognised the decline in crop yields with each passing year, fallowing allowed the land to ‘rest’, and is still a commonly used method, especially among indigenous and subsistence farmers (Paine and Harrison, 1993). The inclusion of cover crops during fallow periods, also known as ‘green fallow’, may help to reduce GHG emissions when compared to dryland cropping systems (Liebig, Tanaka and Gross, 2010).

There is a question over the impact on soil C from other variables in fallow cropping systems, such as soil type, the crops in question, the tillage regime, and nitrogen fertiliser application. Results suggest that a crop-fallow system could further reduce SOC if these variables are not properly taken into account (Halvorson, Wienhold and Black, 2002). Additionally, no net GHG benefit was found from incorporating a rye cover crop during the fallow phase of a dryland cropping system under no till (Liebig, Tanaka and Gross, 2010). C loss still occurs in fallow land, ranging from 320-530 kg C ha^{-1} yr^{-1} in former wheatgrass and mixed prairie sod sites in Nebraska, although losses were reduced under no till compared with ploughing (Doran, Elliott and Paustian, 1998). In terms of land left long fallow (more than ten years), research from former agricultural land in Russia indicates that the properties and structure of soil changes very little in the first two to three decades of abandonment, but C losses can amount to around half a ton per hectare (Romanovskaya, 2006).

The combined evidence seems, therefore, to indicate that the key to bringing benefits from leaving areas fallow is to then incorporate the ‘rest’ times into a wider plan of crop rotation and/or mulching, tillage or organic inputs. There is, however, a lot left under-researched in terms of the time and depth impacts of leaving land fallow.

1.6.3 Tillage regimes

Tillage is a surprisingly modern agricultural revolution, only widespread in Europe from the second half of the eighteenth century. Jethro Tull (1674-1741), revolutionary agriculturalist and inventor of the seed drill, theorised that it could replace some of the traditional required inputs of manure and also reduce the introduction of weeds (Paine & Harrison, 1993).

Abandoning or reducing (‘no till’ or ‘minimum till’) tillage is recommended to reduce net emissions from croplands, because it helps to increase the storage capacity of C and N in topsoil layers. While the efficiency of this process is debated, the consensus is that less is best (Loubet *et al.*, 2011). This is due to tillage increasing erosion potential as well as SOC loss driven by tillage (Halvorson, Peterson and Reule, 2002), and while no till leaves a barrier of surface residue and partially decomposed material which helps to prevent erosion (Doran, Elliott and Paustian, 1998). Most soil C changes take place in the top 30 cm of the soil, that reached by a plough, and therefore the tillage regime can have a representatively large impact on SOC (Doran, Elliott and Paustian, 1998). However, as with any advice this must be tailored to local conditions, as the risk of soil densification from lack of tillage in arid and semi-arid soils may necessitate differing approaches (López-Fando, Dorado and Pardo, 2007).

There is evidence for minimum till improving C content in soil under a variety of agricultural systems and crops. Comparing tilled soil to pasture, Rosell et al. (2010) found a significant decrease in soil C, with pasture also holding higher mineral-associated OC content. Three different cropping systems measured using soil gas chambers showed much greater fluxes from tilled treatments than untilled (Reicosky et al., 1997). A study on three different tillage types showed that with decreasing tillage intensity, the soil was able to sequester more SOC in the annual cropping system (Halvorson, Wienhold, et al., 2002). It is becoming more common to use minimum till and even no till in combination with crop rotation to reduce the losses of organic C and the requirement for inputs (Havlin et al., 1990; López-Fando et al., 2007). While there can be a negative effect of no till on soil structure, this can be offset by using it in combination with a diverse crop rotation (Munkholm et al., 2013).

‘Minimum’, ‘no’ and ‘normal’ tillage are so varied that they don’t have an accepted definition. Trialled on the Game and Wildlife Conservation Trust farm at Loddinton, Leicestershire, as well as a farm in Kent and sites in France and Spain, a Syngenta-funded experiment defined the tillage regimes as:

- No till: Straw disc (lightly breaking up clods of earth) or discing to only 2.5 cm depth before seed drilling
- Minimum till: Light disc (diameter 20-30 cm) or a low disturbance subsoiler before seeds were drilled
- Plough: two passes with the discs and then power harrowed before seed drilling

(Dann, 2019)

There are still unknowns around reducing tillage, such as the effect of no tillage at soil depths which are not directly affected by ploughing, and recovery times in terms of macroinvertebrate and microbial diversity which feed into C recovery, and the relationship with soil N (Jha *et al.*, 2022).

1.6.4 Organic fertilisers, green manure, living mulches and biochar

Tillage was originally designed by Tull to reduce perceived negative impacts of applying manure or organic matter to the soil surface. However, evidence is now supporting a return to older practices such as green manure and cover cropping, as part-solutions to some of the consequences of tillage such as erosion and SOC loss (Paine & Harrison, 1993). Green manuring, cover crops, and organic inputs return SOM into the system in a way that chemical-only artificial inputs do not; in a crop rotation system, there is not necessarily a yield penalty for avoiding these chemical inputs (R. G. Smith et al., 2008) but a lack of SOM is a crucial factor in productivity ("4p1000" CGIAR System Organisation, 2018; Bronick & Lal, 2005; Lal, 2020). However, some level of tillage is likely to persist because of its weed suppression, and also the effect of avoiding tillage on crop yield is heavily dependent on weather (Dann, 2019).

'Green manure' refers to a plant material incorporated into the soil while still green, which can include cover crops and living mulches. A 'living mulch' refers to a second species, usually a legume or forb, planted simultaneously with the main crop, used most frequently in grain production and orchards (Paine & Harrison, 1993), generally ploughed or beaten into the soil the spring after they were sown. The use of living mulches and green manure is recorded from 500 BCE, but its adoption, particularly in Europe, was extremely slow, and not taken up on a large scale until the 19th century; since then it has undergone a rapid decline with industrial farming and artificial inputs, but current soil nutrient depletion is seeing its return (Paine & Harrison, 1993). The modern practice of soil flipping is a more drastic approach to green manure by using farm machinery to overturn soil in chunks up to a metre thick. This is designed to trap the labile C stored in the surface biomass at a depth great enough that it would not be released to the atmosphere, and give the usually deep-horizon soil turned to the surface a chance to accumulate more C from plants and the atmosphere (Schiedung et al., 2019). In New Zealand, flipped soils contained up to 32% labile SOC compared to soils which had not been subject to flipping, confirming SOC stock increase using this extreme green manuring method (Schiedung et al., 2019).

However, it is not necessarily a simple comparison between green manure and soil flipping, as there are varying times over which these benefits are observed, varying depths to which they are effective, and varying persistence of the carbon compounds assimilated. The efficacy of SOC accumulation on flipped soils is on a long timescale – up to 20 years – compared to improvements seen in just six months after green manure application (Puget & Drinkwater, 2001). However, green manure improvements may be limited to the top 15cm of soil, for the first year at minimum and probably longer (Sharma et al., 2017), whereas improvement in SOC at greater depths by flipping can be considered immediate, as O horizon labile carbon is immediately removed from catalysts and air. There is also little information available on how persistent the green manuring must be to be an effectual long-term solution for increasing SOC (Elfstrand et al., 2007).

The application of organic fertiliser (manure) certainly adds to the SOM available for conversion and sequestration in the soil, but it can also increase the emissions of ammonia and nitrous oxides to the atmosphere (Loubet et al., 2011). Additionally, applying liquid forms which contain more labile forms of carbon than solid manure leads to a priming effect whereby the rates of decomposition of C- and N-containing compounds is significantly changed, which may not be conducive to long-term storage (Loubet et al., 2011).

Biochar is another approach to inputs, moving away from the artificial and chemical. A solid product of pyrolysis of biomass under limited oxygen, it is used specifically to increase the stable C content in soil, and can enhance the storage of new root-derived C belowground by up to 20% (Han Weng et al., 2017). The organo-mineral interactions which form microaggregates and contribute to soil structure and the biological processes which accumulate and stabilise SOC are enhanced by the presence of biochar (Han Weng et al., 2017). However, the effects of biochar are, once more, highly dependent on other biotic and abiotic factors including its mean residence time, the priming effect, biochar-induced CO_2 emissions and emissions of other gases such as methane and nitrous oxides, and the distribution of SOC in particles of different sizes. Particle size affects the increase of the SOC, which can be up to 76% in applications of 250-2000 µm size biochar particles (El-Naggar et al., 2018).

1.6.5 Mob grazing

Mob grazing is a practice which takes aspects of the crop rotation, fallow and tillage theories to concentrate grazing, which has been touted as beneficial for both cattle and the environment. It is used to ensure grasslands have short, concentrated spans of grazing and trampling which ensure a long rest period as the livestock are rotated around the site. This does improve species diversity (NFU, 2021), which has been linked to greater soil C. However, research into the carbon sequestration aspects is scarce and, so far, not positive: Russell et al. (2013) found no benefit to SOC content over two years; Guretzky et al. (2020) rejected the concept of high cattle densities improving the litter deposition pathway to SOM formation; and a South African project found that while some improvement in SOC concentration was observed, their projected gains were overestimated and the actual progress was comparable to a variety of other farming systems (Hunter & Mills, 2013). Mob grazing has been found to increase soil compaction and decrease soil aggregate stability (Roberts & Johnson, 2021), leaving it more vulnerable to erosion. Evidence seems to indicate that while there are benefits to mob grazing such as greater fibre content in the grasses (Billman et al., 2020) and greater sward diversity and accompanying biodiversity (NFU, 2021), consistently improved SOC is not one of them, although there is a lack of long-term studies on this practice.

1.7 Agricultural grasslands and carbon

Historic bylaws have been known to recognise what we would today term ecosystem services – “Benefits that are provided by Nature for free” in Székelys, Transylvania, Romania, in 1786 (Molnár et al., 2015), or the statute of 1285 in England that ‘prohibit[ed] fishing for young salmon between the middle of April and 24 June’, and a statute in Cumberland in 1278 whereby ‘weirs on the Eden, Esk and Derwent should have a gap of such size that a sow with her five little piglets can enter’ so as to avoid destroying fry (Canal and Rivers Trust, 2017).

In many areas, including Britain, traditional managements have been used to try to maintain soil stability and productivity, as it has been understood to be the basis for crop health and human survival almost since the dawn of agriculture (Sandor & Homburg, 2017; Turner, 1974). Traditional ecological knowledge (TEK), therefore, has developed strategies over time, which have sometimes become lost in the zeal of intensification, and which some communities are now trying to recover (Berkes et al., 2000). Consequently, many traditional management strategies are being reinvestigated under scientific study. TEK incudes multiple species management, for example the agroforestry practiced for centuries in India (Shankarnarayan et al., 1987); resource rotation, such as crop rotation of barley and wheat followed by peas and beans in highland farms of Ethiopia (Assefa & Hans-Rudolf, 2016); the intercropping system of corn for support, with beans for nitrogen and squash for ground cover, weed suppression and water retention, known as the Three Sisters in Native American agriculture (Herrighty et al., 2021).

1.7.1 Historical use of grassland in rotation

Grasslands have always been recognised for their place within a rotational system, providing some of these ‘benefits that are provided by Nature for free’, for as long as agriculture has encompassed both plants and animals (Dove, 2004; Sandor & Homburg, 2017). Calcareous grasslands are a man-made habitat which did not exist before the neolithic, and did not proliferate in the UK until sheep rearing became a major industry in the fifteenth century (Poschlod & WallisDeVries, 2002). Iron Age grazing in Europe significantly increased the spatial cover of grasslands (Eriksson et al., 2002), and most grasslands in Europe can be traced back to human activity in the Mesolithic-Neolithic transition, indicated by a lack of tree pollen in the record, with enlargement not occurring until the late Bronze Age when scythes indicate the development of hay meadows (Hejcman et al., 2013).

The need for organic matter has been understood in soils for millennia, even if carbon, the crucial element, was not understood as a main driver of soil health (Guttmann, 2007; Lauer et al., 2014). Grasslands have been utilised in nomadic farming, where the vegetation was grazed down to replace a natural scrubbier community, animal dung adding nutrients which persisted in the landscape over centuries, and areas returned to as the land met a new equilibrium of enriched soil (Marshall et al., 2018). In the UK, semi-natural grasslands developed under more constant agriculture, but were commonly fertilised from compost heaps, animal dung, midden and waste, and even charcoal (Hejcman et al., 2013). The key ingredient has always been carbon, and the aim to increase soil carbon is as old as agriculture.

The processes involved in soil carbon sequestration in the UK are similar to those in all soils, be they under forest or vineyard, arid or temperate: the sum of physical, chemical and biological processes which stabilise C from biota; the binding of aggregates by hyphae and roots; the action of soil ‘engineer’ macroorganisms; the compounds and cations from broken-down tissues; and microbial exudates and crust formation. However, climate, humidity and precipitation are key abiotic drivers of the speed and chemistry of these processes (Chai et al., 2022; DeLuca & Boisvenue, 2012; Malik et al., 2019). The UK has a temperate maritime climate with high precipitation and humidity and the warm air of the Gulf stream, combined with a high concentration of peatlands (the UK has over 2 million ha of peat and contains up to 13% the world’s peatlands (IUCN UK, 2018) and high C content soil storing 9838 Mt C, developing over 10,000 years since the last Ice Age (Milne & Brown, 1997). Consequently, UK soil is generally fertile, especially in the lowlands, and has the benefit of being usually well managed and stable since the major deforestation periods from the Bronze Age into medieval times. After a period of stability, soil fertility began to decline from the mid-20th century when compaction, intensification, meadow loss and hedge removal (hedges help prevent soil loss, SOC loss via water run-off, and sequester more C than grasslands per m^2 (Biffi et al., 2022; Lenka et al., 2012)) were accelerated by mass mechanisation and intensification (Dotterweich, 2013).

1.7.2 Grassland C sequestration and productivity

Converting arable land to grassland has been referred to as one of the most effective managements for mitigating GHG emissions from croplands in the long term (Loubet *et al.*, 2011). Carbon sequestration occurs mostly belowground in grasslands, and carbon fluctuations follow land use change (Soussana et al., 2004). As grasslands are generally productive agricultural land, whether whole or part of a production system, farmers and landowners are generally keen to maintain their function. These functions include provision for animal rearing, for ecosystem services such as water regulation, for energy provision in the form of Miscanthus or reeds, or for export to other farms in the form of hay and hay or silage products (Donnison and Fraser, 2016). Maintaining grasslands in a productive state, whilst also taking advantage of any carbon storage facility, is a key part of encouraging management processes which will help to increase soil carbon. As a large part of existing food production systems, grasslands will not be taken out of rotation on any great scale without dietary, social or economic change of the type which would take decades to become established; therefore, any C sequestration benefit they may provide must initially work within the current system.

Grasslands are extremely varied, and therefore their C sequestration potential will undoubtedly be too. Much research has been undertaken on improved grasslands in particular (Conant et al., 2017; Humphreys et al., 2017; Lal, 2010b; Soussana et al., 2010; Whitehead et al., 2018). Take as an example the world's first study of the CO_2, CH_4 and N_2O GHG flux budget of an intensively managed grassland in Switzerland: there is evidence from multiple treatments that gas emissions fluctuated with management and season (Merbold *et al.*, 2014). Managements such as ploughing, harrowing, sowing and fertilising increased nitrous oxide fluxes for up to 14 days after treatment, with large peaks particularly after winter thawing and the addition of fertiliser; however, methane only showed minor fluctuations, and CO_2 flux was minimal until the plants were fully developed, although overall CO_2 was still responsible for 44% total emissions (Merbold *et al.*, 2014). The study showed that ploughing a grassland is the most disruptive and C-costly management, converting the grassland from a carbon sink to a carbon source by way of soil disruption, predominantly by increasing the surface area exposed to the atmosphere. N_2O emissions particularly were highest when the greatest amount of bare earth was exposed before canopy development, but high peaks in emissions followed re-sowing and following the first harvest – highly disruptive activities to soil process stability (Merbold *et al.*, 2014). Light and temperature correlate with ecosystem respiration, linked under more extreme conditions to reduced photosynthetic activity by immature plants with less surface area to absorb as much of the soil gas flux, meaning more of the soil gas is then lost to the atmosphere (Merbold *et al.*, 2014). Methane flux fell to near zero during frozen and very dry periods, and was not statistically significantly tied to any of the management activities or variables (as expected for aerobic soil) (Merbold *et al.*, 2014). A subsequent five-year study of the same permanent grassland, attempting to determine memory effects on GHGs following a return to traditional managements suggested that while significant releases of CO_2 and N_2O followed the immediate return of ploughing, and enhanced emissions were observed for at least seven months following, the CO_2 uptake of the site also increased compared to the pre-management, undisturbed period (Merbold *et al.*, 2021).

1.7.3 Grassland carbon in semi-arid, urban and semi-natural scenarios

Semi-arid grasslands, too, have been under scientific scrutiny with regards to managing carbon content (Denef *et al.*, 2008; Liu *et al.*, 2009, 2017; Chang *et al.*, 2018; Yang *et al.*, 2019). A 2016 study which analysed 30 years of data from semi-arid grasslands in China produced initial falls in SOC from crop to grassland (~5 years), followed by net C gains by 15 and 30 years post-cultivation, with an increasing trend throughout the time period (Liu *et al.*, 2017). Yang et al. (2019) evidenced that grasslands established on degraded or abandoned agricultural lands, which were seeded with a representative community of late-successional diversity, could achieve C storage rates after 13 years which were 70% greater than those in monocultures. The highest supplementary-seeded diversity treatments achieved C storage at 200% greater than in the plots with natural succession with suppressed diversity, and with around 430 million ha of abandoned agricultural land globally, replanting these areas as diverse grasslands could represent a strategic soil C gain (Yang *et al.*, 2019).

Less common, but beginning to become accepted as a possibility for soil restoration, is urban grassland restoration. Urbanisation, the development of dense towns, suburbs and cities, is a severe threat to biodiversity (Klaus, 2013) and to soil accessibility, quality, and restoration to what might be considered a naturally biologically functional state (Moss, 1987; Guilland *et al.*, 2018). In urban-industrial grassland restoration, whereby plants were introduced to highly degraded former industrial sites, green coverage and species diversity were similar to reference grasslands within three years (Kövendi-Jakó *et al.*, 2019); if biodiversity can return in these areas, and links between SOC and species or functional diversity within grasslands can be maintained (Yang *et al.*, 2019), even degraded and brownfield sites can be established as potential carbon sinks in the form of grasslands (Klaus, 2013).

Semi-natural grasslands also have a part to play within the agricultural system, and can be utilised as an important step for soil health and recovery (Soussana et al., 2004). The definitions of grassland types for the purposes of this thesis is given below in Table 1.1 with definitions from Champion (2022).

Table 1.1. Definition of UK grassland categories within this chapter

Grassland	**Definition** (Champion, 2022)
Natural	Grassland that is the climax vegetation, i.e. where the climate or natural conditions prevents scrub or trees establishing. Unmanaged by human activity. Very rare in the UK and, by definition, not found on any land used for grazing or productivity.
Semi-natural lowland	Grassland where cutting, grazing or burning prevents scrub or trees becoming established, but is otherwise unaltered by human activity such as drainage or fertiliser application. Generally more fertile than semi-natural upland, with a range of uses.
Semi-natural upland	Grassland where cutting, grazing or burning prevents scrub or trees becoming established, but is otherwise unaltered by human activity such as drainage or fertiliser application. Generally less fertile than semi-natural lowland with historical rough grazing, sheep-dominated.
Semi-improved	Grassland that has had some agricultural improvements made to it, such as drainage or some fertilisation, but where botanical biodiversity is maintained through a mixed sward.
Improved	Grassland that has been managed to increase its productivity, usually by a combination of drainage, fertilisation, herbicide use, ploughing and/or reseeding.

With the predicted increases in wetter weather across much of the southern and western hemispheres under a 1.5° warming scenario, semi-natural grasslands are threatened by management practices with impacts on biodiversity and soil health, (Napoleone, Giarrizzo and Burrascano, 2021) with follow-on impacts on carbon storage capacity. The global range of semi-natural grasslands means that the risks associated with a changing climate can range from desertification, such as in the Tarim Basin in China (Liu *et al.*, 2016), to the loss of permafrost semi-natural alpine grasslands, such as the Qinhai-Tibet corridor (Jin *et al.*, 2008). Desertified ecosystems lose soil carbon as well as structure and resilience (Lal, 2002) and the loss of permafrost leads to significant water loss, soil erosion, and vegetation destruction (Jin *et al.*, 2008). Semi-natural grasslands are found across many biomes and climates, from the Arctic to sub-Saharan, supporting a wide range of biodiversity and ecosystem services including primary production, biodiversity, pollination, and nutrient cycling and retention (Villoslada Peciña *et al.*, 2019). Therefore, these losses must be seriously considered and management adjusted where it will make a positive impact (Allard *et al.*, 2007). From established research, a much more restricted approach to certain management strategies could be espoused, such as a reduction or prevention of ploughing, and a return to longer or more frequent periods of grassland establishment within an agricultural rotation. These could be important tools in minimising gas flux from current grassland sources of carbon. Even moderately intensifying existing nutrient-poor permanent grasslands could balance sequestration with production and improve the cost-benefit ratio with regards to carbon (Soussana et al., 2010). With minimal disruption to existing systems, these strategies could indeed form a crucial sink for atmospheric carbon.

1.7.4 Semi-permanent or temporary leys

Almost all research published to date focuses on improved or semi-natural permanent grassland (Bell *et al.*, 2011; Graux *et al.*, 2011; Muhammed *et al.*, 2018). Less is known about the effect of short duration leys on soil C (Soussana et al., 2004), particularly those established for five years or less. Very little is known about soil C storage processes in the short term at all, and the five-year mark for grasslands is important in British and European systems in particular, as these are already subsidised under the Common Agricultural Policy (CAP) framework. In the UK this is distributed by Natural England in the form of stewardship payments, which is currently in the process of transitioning to Environmental Land Management Schemes (ELMS).

In the UK, temporary grasslands make up 20% the total utilised agricultural area (UAA) (DEFRA, 2020), so this is a large proportion of land area which may be being promoted for its soil C benefits, among other attributes, when the effect of the short-term establishment of grasslands is far from well-established (Hirsch *et al.*, 2017). Temporary grasslands are financed under basic payments and more diverse leys housing a greater variety of flowering species, including buffer strips around ponds, watercourses and field boundaries, are already funded under environmental stewardship schemes (Environment Agency, 2013). Grasslands are often used in rotation to sustain nutrient concentrations and soil fertility (Jarvis *et al.*, 2017). Grasslands are also touted as a cure for soil erosion through reduced management of field corners, maintaining grass, reducing inputs or creating rush, legume or herb-rich swards (Environment Agency, 2013), and various managements at three intensity levels to increase soil C, OM and nutrient content are incentivised by the UK government (DEFRA, 2022a). But there is a risk that funding is being promoted for temporary grasslands which do not bring a carbon benefit with a lasting effect. On the other hand, there is a possibility that in paying for a temporary grassland to be established due to its other recognised benefits, such as biodiversity gains, a further advantage in soil C could become evident. In this case, temporary leys could be further incentivised to increase their prevalence in the landscape for a wider social benefit under the various schemes, strategies and targets the UK has in place for both soil and emissions improvements. Increasing the established range of benefits of leys to include a long-term carbon investment may attract more incentives for farmers to keep temporary leys in rotation, or at least prompt the removal of disincentives for taking leys out of rotation (for example, paying a competitive price with other forms of land use, or matching carbon credits).

1.7.5 Individual species attributes to ley carbon

A key aspect of grasslands which has not been established particularly well are the attributes of the species, or species mixes, which populate them, and the impact of these species on soil carbon (Skinner *et al.*, 2006; De Deyn, Cornelissen and Bardgett, 2008; Jones, Nguyen and Finlay, 2009). There is particularly little research on these in temporary ley systems. Species diversity is known to positively impact soil C content, but information on specific species is scarce (Steinbeiss *et al.*, 2008; Pausch and Kuzyakov, 2018; Yang *et al.*, 2019). Deeper rooting plants have been connected with changes in C, nutrient and microbial communities, but few individual species examined, especially plants which are not crops (De Deyn, Cornelissen and Bardgett, 2008; Kell, 2011; Hafner and Kuzyakov, 2016). Legumes have been in rotation for at least 8,000 years, because humans have long linked them to improved crop production and improved soil fertility, even before their nitrogen-fixing qualities were understood (Zohary and Hopf, 1973). Nitrogen concentration has a positive relationship with soil carbon (Tian, Dell and Shi, 2010; Guan *et al.*, 2016), because N inputs allow a microbial, rhizomatous or plant root community belowground to synthesise more atmospheric carbon which consequently becomes exudates or dead tissue belowground (Phillips *et al.*, 2012; Kaštovská *et al.*, 2015). Currently, grasslands established under Natural England schemes must have at least 5% legume seed in the mix, but other than that, there are no prescriptions for plants known to have a particular effect below ground. This is because there are few plants commonly found in grasslands which have a known and desired effect on any soil parameter that has so far been incentivised, with the exception of legumes (Skinner *et al.*, 2006; De Deyn, Cornelissen and Bardgett, 2008) While there are a several soil strategies and managements that are encouraged under environmental land management and stewardship schemes, including under the Sustainable Farming Incentive which will be rolled out across the UK in 2023-24, too few functional traits or species have been investigated individually to incentivise their establishment (Skinner *et al.*, 2006; De Deyn, Cornelissen and Bardgett, 2008).

1.7.6 Depth fluctuations in soil carbon

The depth aspect of soil carbon is also not particularly well understood, especially on the short timescales of temporary leys, as most studies are concerned with impacts over ten years or more (Alcántara et al., 2017; Bork et al., 2020; Havlin et al., 1990). Carbon is assumed to have higher influx but also efflux into the top layer of soil, particularly due to root density and exudation, which therefore interacts with biota and surface processes more than deeper soil (De Moraes Sá et al., 2014; de Oliveira et al., 2016; Mcsherry & Ritchie, 2013; Rasse et al., 2006; H. Wang et al., 2020). Surface processes appear to have little impact on soil carbon below the first 15 cm (Whitehead et al., 2018). However, as previously discussed, microbial and plant-mediated effects can influence the compounds and accessibility of C when it enters the soil system (Cotrufo et al., 2013; El-Naggar et al., 2018; Six et al., 2002).

It would seem logical that the storage mechanism of carbon at depth would relate to rooting depth of the plant community, as deeper roots may influence the surrounding soil at greater depths, both physically and chemically (Gregory, 2006; Jarvis et al., 2017). Additionally, plant species often influence the microbial communities around their root system by means of their exudates and other physical and chemical changes they bring to their immediate environment (Gregory, 2006; Karlowsky et al., 2018). The microbial and fungal community is a particularly important part of the soil nutrient cycle (Carrillo et al., 2011; Karlowsky et al., 2018; Potapov et al., 2017; Qi et al., 2018; Storer, 2013).

It may be difficult to tell direct root effects on soil C apart from the indirect effect that deep rooting species have on the water content of the soil, as water may modify microbial community structure or influence the microbial community synthesis of C compounds (Sainju *et al.*, 2017). Conversely, a lack of water may also provide more SOM in the form of dying tissues (Sainju et al., 2017). There is a distinct lack of studies focusing on root morphologies or functional groups with regards to soil C and the microbial community (Sainju et al., 2017).

In a desert ecosystem, the legume *Prosopis glandulos*a (mesquite) was associated with functional root symbioses down to 4 m depths, close to the water table, and symbiotic microbes were greater in density at depth than at the surface; however this is likely due to the dryness of the soil above the water table, and not simply a root characteristic (Virginia et al., 1986). Legumes have also been linked to deeper C storage in semi-arid soils, but on a timescale of seven years (Guan et al., 2016).

A review of studies from New Zealand, however, discovered the opposite effect of roots on soil carbon: that a greater mass and length of root in deep soil layers under grazed pasture was associated with lower soil fertility (Dodd et al., 2011). Poirier et al. (2018) found that while root length and diameter did not relate to C stabilisation processes, root depth distribution is the most important trait driving C root storage in the subsoil by way of increased rhizodeposition, root chemistry, and interaction with minerals and metals in the soil. This shows the relationship between plant root and soil C depth patterns is far from established and that much more research, with comparable methods, needs to be published to enhance current suggestions with regards to deep rooting species and their influence on soil C.

1.8 Purpose of thesis

This thesis aims to address some of the gaps in the literature indicated throughout the introduction, exploring the effect of commonly grown ley species on limited timescales and throughout a range of soil depths on soil carbon. In the global context of requiring an increase in food production to meet the population's needs during climate breakdown, existing resources must be fully utilised to optimise soil health and climate benefits which the existing system may offer. Facing current challenges of sustainable farming with rising demand, changing climate, and a biodiversity crisis, we are in a transitionary period where new technologies, approaches and legal or logistical frameworks are evolving to meet these challenges and research is being conducted into improving methods or exploring new options for food supply and security (Harvey & Pilgrim, 2011; Howden et al., 2007; Ponisio & Kremen, 2016). Agriculture has already seen this at many points in history, from the development of livestock and arable farming, to the adoption of farm machinery, now even to technology such as insect farming, vertical farming, and 'lab meat'. During this transition period to the systemic change needed to support a farming future, we can help to meet challenges in soil health and atmospheric CO_2 concentrations with the land use, technology and systems already in place.

As a knowledge gap was identified around the short time period of ley establishment, the two greenhouse experiments were designed to help fill this by approaching from the perspective of a one-year (WG2) or a two-year (WG1) ley. The experiments were designed with analysing the depth effect down to 1 m as an integral part, given the lack of depth data in the literature body. The laboratory analyses were conducted to establish not only total C, but fractions by pyrolysis, to address the questions regarding the speed of development of labile and recalcitrant C pools and whether these are related to depth. A variety of species, functional group and rooting depths were included to examine links between species and depth effect on soil carbon. Analysing an existing ley experiment at the Halstead experimental farm in Leicestershire with the benefits of in situ conditions including grazing and legacy effects will help to bring the effect of these common practices on soil C to light. It is important in these experiments to take account of a depth profile beyond the usual plough depth to give detail on the movement of carbon both through fractions of lability and, possibly relatedly, the soil horizons. And the final important aspect when designing these experiments was to take account of species, functional group and/or rooting depth to attempt to tie these aspects of morphology, chemistry and function to the effects in the associated soil with regards to soil C.

The purpose of this thesis is to contribute to the store of knowledge on how the plants and timescales commonly used in agricultural leys support carbon storage in the soil, and in what fractions and depths. Across four chapters and experiments, I explore differences in cover crop species and functional groups, their impact on total and pooled carbon across depth and time, and the impacts of grazing on grassland soil emissions.

In the single-species pots in the Walled Garden 1 mesocosm, I hypothesise that:

- There will be a positive relationship between plant species diversity and carbon concentration
- There will be a positive relationship between functional group diversity and carbon sequestration
- Sequestration of different carbon fractions of reactivity will correlate to different depths

In the single- and multi-species pots in the Walled Garden 2 mesocosm, I hypothesise that:

- Gains in soil C will be greater over the two year mesocosm (WG1) than the one-year mesocosm (WG2)
- There will be an effect of seasonality in soil C fluctuations during the life cycle of the ley mesocosms
- Low-carbon soil will shows a greater improvement in SOC stocks than C-rich soil
- Plants grown in low-carbon soil have deeper rooting systems than those in C-rich soil

In the Festulolium/Donata ley experiment in Halstead, I hypothesise that:

- Grass cultivars show differences in the C content of supporting soils
- Differences in SOC are driven by differences in root biomass
- Deeper rooting cultivars will store a greater proportion of their C in more recalcitrant forms
- Shallow rooting cultivars will store a greater proportion of their C in more labile forms

In the meta-analysis across N2O, CH4 and CO2 emissions from different grassland-grazing systems, hypothesise that:

- Britain holds more livestock than is sustainable for maintaining soil organic carbon levels
- Equivalent sheep LSU is more climate-sensitive than cattle LSU
- Upland pastures are more sensitive to changing LSU than lowland pastures
- Intensive approaches, already inherently high climate impact, are least sensitive to increasing LSU

2: Methods

2.1 Introduction to methods

The structure of this thesis is based around an *in situ* experiment and two *ex situ* experiments, which fundamentally revolve around the sampling of soil and its processing for basic parameters, nutrients, and carbon content from experiments on several species, functional groups, and/or species mixes. Because of the repetition of several methods within this thesis, this chapter will be referred back to in later chapters.

Establishing the *ex situ* experiments will be explained in Chapters 3 and 4, and the establishment of the *in situ e*xperiment further explored in Chapter 5. A brief explanation will be given here. Chapter 6 is a review and the methods are also given here, although further detail is in Chapter 6 and Appendix 1.

The *ex situ* experiments were established in the Walled Garden of the University of York (53°56'43.0"N 1°02'56.8"W), ley mesocosms referred to as WG1 and WG2. WG1 was established with five replicates of six single-species plant pots and five controls, planted in high-carbon content peaty topsoil in a grid pattern to minimise edge effect in February 2020. WG2 used single, double, and triple species combinations planted in low-carbon content mineral soil in two lines to minimise shade effect in July 2021. Each were sampled at three-to-six-month intervals, at four depth points spanning 10 to 90 cm, to glean insight into the effect of the plants on carbon over time and at varying depth.

The *in situ* experiment in Halstead, Leicestershire (52°38’51”N , 000°52’57”W) was established for four years, and I sampled it in its fourth year, before it was sprayed off to be planted into herbal ley. The experiment comprised three replicates each of six cross-breeds of Festulolium grasses, creating 18 strips buffered by ryegrass either side. The plots were fenced across in the middle to also create a grazed vs. ungrazed comparison. This experiment was grass-focused rather than the herbal component of WG1 and WG2, with only the 5% clover cover in each patch to meet the governmental recommendation.

The purpose of each experiment was to link carbon concentration and fraction, the response variables, to the predictor variables of depth, time, or plant species. Both predictor and response variables could also be linked to related mediator variables such as pH, bulk density, soil water content, volatile organic acid concentration, or nutrient content.

While the specific set-up of each experiment will be expanded on in their relevant chapters, below are the sampling and laboratory processing methods for the analyses I conducted on soil.

2.2 Field methods

2.2.1 Study sites preparation for Walled Garden 1 & 2

The first and second mesocosms are referred to as WG1 and WG2 after their location in the University of York Walled Garden, Heslington, York, UK (53°56'43.0"N 1°02'56.8"W). The mesocosms were outside rather than in a greenhouse to take advantage of rainwater as leys would *in situ*. 35

pots for WG1 and 40 for WG2 were made using 1 m lengths of opaque, round, rigid PVC pipe, of 150mm internal diameter and 155mm external diameter (source: https://www.ducting-online.co.uk/pl150-rd-round-rigid-pipe-c2x14449876). The base of each 1 m pipe was bonded with high strength adhesive to a plastic plant saucer with a diameter of 165 mm (source: https://warboyshardware.co.uk/grow-saucer-terracotta-165cm-p-1022.html) to form a 1 m-long plant pot. The long pots were drilled with holes of 20mm diameter, with the centre of the holes at 100 mm, 300 mm, 600 mm and 900 mm from the top of the pot.

Two tons of multi-purpose topsoil was initially sourced from East Riding Horticulture Ltd. During WG1, the soil was found to be carbon-rich (~ 10% C). Therefore, soil was sourced for WG2 from the Escrick Park Estate. The soil was sandy loam, ~ 2% C, formerly used to farm turf and therefore depleted of C and nutrients.

Soil for each experiment was shovelled into buckets and barrows. The East Riding Horticulture Ltd topsoil for WG1 was pre-sieved to 2 mm, and presumed homogenous. The Escrick turf was shovelled twice (from van to buckets/barrows and barrows to long pots) and large stones removed during the shovelling and filling of the pots. No fertiliser was applied, as the purpose of the experiments was to determine effect of plant species alone on soil C and nutrient levels, and not the impact of additives.

In WG1, the crowns were supplied from a controlled allotment sown on cultivated garden soil. There were no inputs, including herbicides or pesticides, onsite and weeds were hand-pulled. The crowns were seeded from Cotswold Seed supplies. The *Plantago lanceolata* (ribwort plantain) cultivar was ENDURANCE, the *Chicorium intybus* (chicory) cultivar was PUNA, and the *Achillea millefolium* (yarrow), *Trifolium pratense* (white clover) and *Phleum pratense* (timothy) unnamed certified seed. Dandelions were collected from cultivated garden ground in Prudhoe, Northumberland. The crowns were transported with the roots wiped clean, retaining a soil inoculum, wrapped in damp kitchen roll, and wrapped in a plastic food bag to retain moisture. The crowns were then planted directly into the long pots, prepared with pre-drilled holes and filled with topsoil. Crowns were separated into 'small', 'medium' and 'large' plants as there was no facility to weigh them accurately enough in the field. Plants were grouped to contain approximately the same weight of plants in each pot and then planted in the long pots.

For WG2, seeds were sown into small pots using leftover nutrient-rich WG1 soil (unused for the WG1 experiment). The seeds were sown 4-5th May 2021. These initially yielded a germination rate of about 30% following three weeks of bad weather in, so a second round of planting was carried out on 26th May 2021. Throughout, when necessary, the plants were watered. These late May plants germinated successfully.

The WG2 plants were transplanted from the small pots of SOM-rich topsoil into the 1 m-tall pots of C-poor soil for long-term establishment on 12th July 2021. Before allocation, the plants were uprooted, keeping the roots as intact as possible by first loosening the soil. As much soil as possible was then removed from the root matrix by brushing out delicately with fingertips, which leaves the inoculum of current root associates intact. Plants again were grouped into small, medium and large to allow similar weights of plants to be evenly distributed.

In both WG1 and WG2, pots were numbered and the species or species mixes assigned to each number were recorded. WG1 was arranged in a rectangle grid system, with five replicates of each species randomly distributed to randomise shade and effect and the effect of reduced insulation for the outer pots. WG2 species and species mixes were also randomly distributed, with five replicates of each treatment assigned a code number in order, and these numbers fed through a random number generator to give the placement of the pot. WG2 was arranged in two lines, rather than a grid, because of ease of sampling. This also minimised the shade effect. Loft insulation inserted in to bin bags for waterproofing was bound around the outside of the two lines to reduce the lack of insulation around the pots, which the plant roots would experience if they were at subsoil level rather than in raised pots.

2.2.2 Core sampling

The soil was sampled using a plastic 20ml syringe with the end sawn off, in order to give a small corer with visible volume indicators. The syringe, with the plunger drawn out to its maximal extension, was driven horizontally into the pre-drilled holes in the metre-high plant pots in the WG experiments, or the pit sides for the Festulolium experiment, at depth intervals of 10, 30, 60 and 90cm. The volume of the soil core in millilitres was noted. The soil core was ejected with the plunger then placed into a pre-weighed plastic Ziploc bag, and labelled with the experiment (WG1, WG2, or Festulolium), the pot or plot (to reference the concordant plant species), the sample date, and the core volume in millilitres. The soil was stored in a cool box with ice packs until the end of the sampling period, when it was either frozen immediately (due to restrictions caused by the pandemic) or refrigerated overnight when restrictions were eased and the processes requiring fresh soil could be conducted the following day.

There were two differences between collecting cores from the WG experiments and the Festulolium field: firstly, in the WG, samples could be collected through pre-drilled holes in the pots, whereas in the Festulolium field the size of the plots, the presence of another team studying grazing effects, and reaching one metre depths in a compacted field required the use of a front loader to dig pits; secondly, was that the compacted nature of the soil in the Festulolium field necessitated the aid of a mallet to insert the corer.

2.2.3 Root sampling

At the experiments' close, three out of five replicates across both WG experiments were chosen at random to be destroyed for root analyses. Three were destroyed rather than all five replicates to give a number which would have statistical strength in analyses, but reduce the number of days which would be given to this time-consuming task. The metre-tall pot was marked to where the soil surface actually was (given sinkage over time) and then the top 2 cm of soil was also marked as the depth to which leaf matter, other debris, and plant tissue which is not roots would remain at high concentrations. The pot was sawn off at this point using a hacksaw. The pots were then sawn into 10 cm sections from that -2cm surface point to the base. Given any final section would usually be <5cm, they were combined into 70+ cm or 80+ cm depth sections rather than creating a new section for the lowest pot depth.

Due to soil differences, WG1 and WG2 were sorted for roots in different ways. An effective way to extract roots from the very fine, silty and nutrient-depleted soil of WG2 was to empty the core into a bucket, crush it up, and pour in water to cover the sample. The soil was then sorted through by hand in the water, as roots floated but the soil did not. The soil was disturbed for a period of around 15 minutes while all possible root extraction took place by hand. Then the water would be emptied from the bucket through a 2 mm sieve to capture any remaining fine roots, and the silty soil tipped away. All roots were placed into plastic bags labelled with the experiment number, the pot number, the species, and the depth marking.

The soil of WG1 necessitated a different approach as it was airy, floating, and did not break up as effectively. This required sorting through dry by hand. The cores were tipped into large trays, broken up into chunks, and sorted through by hand to retrieve any roots. Small chunks of the soil, once thoroughly checked and all visible roots retrieved, were disposed of to aid progression. Once again, all roots were placed into plastic bags labelled with the experiment number, the pot number, the species, and the depth marking.

2.2.4 Laboratory methods

Water

The water content (%) was given by taking a subsample of ~3g FW soil and drying it in an aluminium foil crucible for 72 hours at 70^{o} in a Genlab oven. Weights were taken to 0.001 g accuracy. A subset of the crucibles was weighed at 24, 48 and 72 hours. Although no significant water loss was observed post-48 hours, all samples were left for 72 hours to ensure a constant weight. 70^{o}C ensured carbon-containing compounds were unlikely to react, but it was hot enough to prevent decomposition by active microorganisms.

BD

The weight of the plastic bag and label was subtracted from the total weight of bag, label and soil, and divided by the millilitres of soil (weight over volume), to give bulk density (BD). Volume of soil cores was taken to the nearest 0.5 ml and weights to 0.001 g accuracy. The BD of the soil will have some unavoidable margin of error due to the presence of small stones, or small differences in the weight of bags. Fresh bags were weighed rather than the actual bags containing the cores due to the impracticality of completely removing all traces of soil and water, especially for those bags that, due to limited laboratory access during the pandemic, were stored in the freezer around extra moisture. Ten bags of each kind that were used across the experiment were weighed and a mean weight used in BD calculations.

pH

A measurement of pH was taken using a pH meter (Jenway Ltd, Felsted, Essex). A sample of 1g FW soil was added to 10ml deionised water, vortexed for 30 seconds, and the pH read. A subset of 20 samples were put into solutions of both 1:10 soil in deionised (DI) water and 1:10 soil in 0.1 mol KCl, and the pH readings compared. The March 2020 and June 2020 soil samples showed no significant difference between the pH readings taken in DI water and 0.1mol KCl, and therefore the DI water readings were used and the rest processed with DI water only, as these DI+soil solutions could then be retained for analysing the nutrient contents. However, the samples from September 2020 and all subsequent quarters showed a statistically significant difference in pH between those processed in DI water and in 0.1mol KCl. In this case, the KCl readings were used, as KCl is a more reliable solvent.

C and N

The dried soil was processed for total C and N content, and C:N ratio, using the VarioMACRO Cube (Elementar, Langenselbold, Germany). Dried soil was homogenised and ground into fine powder with a pestle and mortar, before 0.05g was decanted into standard foil cups to be processed. The VarioMACRO Cube has a deviation of <0.5% relative with 100 mg glutamic acid. Calibration is multiple point and curve adjusted, in two sections. With each run of 30 samples, after any shut down longer than 2 hours, the machine was conditioned with two samples of glutamic acid, two or three standard blanks, and one standard of soil of known C content. Factor determination adjusted the calibration to atmospheric conditions of the timepoint of the analysis. Leak tests were conducted before each sample batch to ensure gas tightness of the VarioMACRO and ensure complete combustion. The majority of the C:N analyser batches were run by Rebecca Sutton due to training restrictions related to Covid-19, although training was completed in 2021.

Nutrient concentration

The 1:10 ratio of FW soil in DI water was used to measure the nutrient load of nitrites, nitrates and phosphates in a ICS-2000 ion chromatograph (IC) (Dionex, Sunnyville, California). 1 g of soil was added to 10 ml DI water, vortexed for 30 seconds, and centrifuged at 3500 rpm for 15 minutes. The supernatant was filtered through 0.2 µm disposable filters into fresh falcon tubes. The nitrate, nitrite and phosphate values were recorded in µm ml^{-1} soil solution, therefore the water content (%) for each sample was used to convert the results into µm nutrient g^{-1} dry weight equivalent (DWE) soil. These samples were run on the IC by Dr Matt Pickering due to restrictions on time and training related to Covid-19.

Pyrolysis for OM

C was also measured in fractions by pyrolysis. Initially, sucrose standards were run. Calculations were run to determine the amount of C which could be present in the furnace at one time, using the dimensions of the furnace, ratio of air gases, and ensuring that an excess of O_2 would be present when burning the samples. A sample of 0.2g dry soil, the lowest weight represented in the literature (Heiri et al., 2001; Lowther et al., 1990), used to maximise efficiency with the O_2:C ratio, was fired in the muffle furnace (Carbolite AAF 1100, Hope Valley, England). Intervals of 250, 325 and 550^{o}c were run, adhering to the three main peaks of organic matter weight loss observed in thermograms, indicating fractions C at different labilities (Rovira & Vallejo, 2000). After three hours at each temperature, the crucibles were re-weighed. At 550^o, all organic C should be burnt off (Rovira & Vallejo, 2000). To attempt to better define the 'pools' of C, referenced above in the introduction further, my experiments have focused on pools of reactivity according to three peaks of loss on ignition, which are generally seen at 250^o, 325^o, and 550^{o}C, and compared these soil depth to ascertain whether reactivity pools can be associated with depth and whether lability can be partially explained by proximity to the surface.

GC-FID

Samples were analysed for volatile organic acids (VOAs) using 1:10 ratio of FW soil to 0.1mol KCl. The solution was centrifuged at 4000 rpm for 15 minutes, filtered to 0.2µm, 1ml transferred to a sterile glass vial and acidified using 7.5µl of orthophosphoric acid. VOAs were measured using a gas chromatography flame ionisation detector (GC-FID) (HP 5890, Bracknell, UK). The initial set-up, maintenance and troubleshooting of the GC-FID was managed by Dr Yumechris Amekan, as training could not be undertaken due to Covid-19 restrictions. Several sample runs were also subsequently run by Dr Amekan and Luna Pulford MSc due to restrictions. The standard was volatile free acid mix (CRM46975) diluted with MilliQ water to concentrations of 10 mM, 5.0 mM, 1.0 mM, 0.5 mM, 0.1 mM and 0.005 mM. Three washes in pure water were conducted between every five samples to reduce the possibility of cross-contamination. Samples were re-run every 10 samples to ensure accuracy in VOA readings. Acetic, iso-butyric, iso-pentanoic, hexanoic, heptanoic, octanoic and nonanoic acids were detected. Heptanoic, octanoic and nonanoic acids were discounted as most samples showed zero or negligible values that were likely noise.

Roots

To include the aspect of root presence at each depth, to correlate with any plant-mediated depth effect, three out of five pots across the two walled garden experiments were destroyed, as above. In the laboratory, fresh weight roots were tipped into a 2 mm mesh sieve, rinsed thoroughly to remove any remaining soil, and towel-tried. They were packed into weighed envelopes labelled with the experiment number, the pot number, the species, and the depth marking. The pre-drying weight of the envelope was recorded. The roots were dried for 168 hours at 70^{o}C, to remove any moisture without degrading the roots. The post-drying weight of each envelope was recorded to give a dry biomass of roots at each depth interval for all three replicates of each species/combination across both experiments.

3: Grassland plant-mediated effects on concentration of soil carbon in a controlled isolated-species experiment (first Walled Garden experiment)

Abstract

Grasslands account for over half of the agricultural area in the UK, 21.6% of which are established for less than five years. These grasslands are subsidised through stewardship schemes for their perceived benefits to biodiversity. An option for integrating this large area of land use into measures to meet soil health and GHG reduction targets could be explored, if we find evidence of temporary grasslands providing significant increases to soil C and soil health.

With a single isolated species occupying each pot, six herb species were established in a mesocosm with controls was established incorporating herbs and a grass common in leys, covering different functional groups and rooting morphologies (*Taraxacum officianale, Plantago lanceolata, Phleum pratense, Cicorium intybus, Trifolium repens*, and *Achillea millefolium*). The experiment was designed to test the following hypotheses:

1. The relationship between plant species and carbon concentration
2. The relationship between functional group and carbon sequestration
3. The sequestration of different carbon fractions of reactivity in relation to soil depth
4. Assess these over a 1-2.5 year timescale, as mimics a temporary ley
5. Seasonality in soil carbon fluctuations during the life cycle of the ley

Water is a good predictor of total C, related to direct and indirect processes of C assimilation including influencing microbial activity, soil stability, bulk density and primary production.

Mid-lability C was related to pH, likely influenced by the process of humification which produces acids and breaks down OM into C compounds.

N is driving differences in mid-lability and recalcitrant C but not in active labile C or total C.

Cicorium intybus (chicory) can adapt its rooting strategy to the C and nutrient levels in its growing medium but does not have an impact on C, whether total, deep, or recalcitrant. *Achillea millefolium* (yarrow) is the only species significantly driving differences in any fraction of C. Total LOI is influenced by yarrow but no other species, and not whether the soil is control (bare) or covered. Yarrow is linked to changes in labile C and total LOI, indicating a significant impact on soil C. No other species had a significant influence on soil C.

Differences in labile SOC are mostly driven by changes in the top 10 cm soil. There is a relationship between lability and shallowness in WG1, but no evidence to establish a link between recalcitrance and depth. Mid-lability C also linked to 10 cm depth. No depths other than 10 cm showed any link to total, recalcitrant, or fractionated C.

Time progression varies in its predicting strength for soil C. Total LOI responds to the month in which the sample was collected, indicating a seasonal change or change over time driven mainly by labile C. No relationship between root biomass and C was observed. No relationship between root biomass and depth was observed.

Funding C leys of these species is not an effective use of finance directed solely towards improving soil C concentrations. Future research should incorporate plant growth, microbial abundance and diversity, and depths below 20-30 cm as there are data gaps.

3.1 Introduction to WG1

Grasslands, including herbal leys, account for 54.4% the total utilised agricultural area of the UK (Nafilyan, 2015). 3.5 million hectares are permanent, compared to 756,000 hectares of grasslands which have been established for less than five years (DEFRA, 2021b). Over 95% of the UK land carbon stock is located in soils which are subjected to a range of land uses and land use change (Ostle et al., 2009), one of the largest contributors to which is agriculture. Despite this, accounting of the net changes to ecosystem services under land use change is often incomplete or underestimating the benefits, either social or environmental, and consequently, economic, that the change will bring about (Bateman et al., 2013). Accounting for the carbon changes under temporary grasslands as a temporary or rotational land use change, despite its importance with relevance to national and international commitments to carbon reduction targets, is underrepresented in decision-making and policy (Bateman et al., 2013; Smith et al., 2010).

The soil of temporary grasslands stores more carbon than those of croplands in the UK, although less than permanent grassland (Smith et al., 2010). The release of carbon from the soil supporting grasslands in the UK is in contradiction to the provision in the Marrakech Accords of accounting for carbon in carbon sinks, which was directly aimed at both forestry and agriculture, to contribute towards UK commitment to the Kyoto Protocol targets of emissions reductions (Soussana et al., 2004). If the national and international commitments are to be met, every tool in the arsenal must be utilised to incrementally break down the UK contributions to global greenhouse emissions, including utilising current grassland systems and researching ways to improve them, not just to prevent further release of carbon-containing compounds, but contribute to future carbon sequestration by utilising the potential of 'refilling' a depleted carbon store.

Even without atmospheric C, the C potential for this lost niche is crucial given that C is the fundamental building block of harvest. Our food supply is reliant on a soil which contains a healthy and sustainable amount of carbon, championed by the French COP21 initiative '4 per 1000' for restorative and regenerative agricultural practices for our soils to increase soil carbon by 4% each year ("4p1000" CGIAR System Organisation, 2018). 75% global soils are already degraded in some way and this affects our ability to feed our growing population ("4p1000" CGIAR System Organisation, 2018); C is crucial not only as a building block of the compounds that make up the food we eat, but also in maintaining the soil itself as a physical medium for plant growth, and its delicate system of water and nutrient assimilation for sustainable fertility (Beddington, 2010).

While the effects of permanent grassland on soil C are well established (Conant et al., 2017; Hörtnagl et al., 2018; Liu et al., 2017; Mahanta et al., 2020; Medina-Roldán et al., 2012; Soussana et al., 2004) there is a gap in the knowledge regarding temporary agricultural leys. Given the area that temporary leys occupy, and that the specific locations shift on a scale of 1-5 years, ascertaining their contribution to the carbon balance is important. Given that the majority of carbon storage in a grassland will occur belowground, into the soil (Soussana et al., 2004), the fact that the soil is physically disturbed when the fields return to agricultural rotation requires consideration in the long-term plan for carbon balance.

Likewise, all grasslands are individual and comprise a number of species, ranging from one or two in highly intensely managed lowland pasture (for instance, *Lolium perenne* with 5% *Trifolium repens*) to up to 40 plant species per square metre in a species-rich floodplain wildflower meadow (Sussex Wildlife Trust, 2022). However, most grasslands in the UK are improved or semi-improved, with 98% meadow loss over the 20th century (Sussex Wildlife Trust, 2022). These species-poor grasslands still retain a C benefit, although biodiversity is known to increase carbon storage potential (Mellado-Vázquez et al., 2016; Yang et al., 2019). Natural England also incentivises grasslands with greater species diversity (Natural England, 2015). For these reasons, plant diversity and functional diversity are important factors in the experimental design.

There is some debate over the steady state or pooled state of carbon in our soil ecosystem, as discussed in Chapter 1 (Andrén et al., 2004; Cook et al., 1998; Fang et al., 2014; O'Rourke et al., 2015; Paul et al., 2006; Pausch et al., 2016). Likewise, it is not fully understood how pools of reactivity (the lability of stored C and how it is incorporated into these compounds) may or may not be related to the depth at which the compounds are produced and/or stored (Piccoli et al., 2016). However, it is understood that because of the depth of disturbance, i.e. by a plough or livestock, which generally would disturb the top 20-30cm of the O and A horizons, compounds which are stored below this reach are likely to escape reacting with atmospheric gases, even if they can then be reached by plant roots and used to produce tissue. However, whether there is a causal relationship between depth in the soil and the presence of less reactive compounds is not well understood.

To assess the effect of plant species diversity, including herbs and legumes, on soil carbon in a temporary ley, this experiment was designed. The key aspects to include were not only species and functional group diversity, but to measure it on a timescale over which a ley would be sown and then ploughed out, which is generally 1-5 years, although 'temporary' can be defined as up to 10 years under Natural England guidance. Depth would also be an equally important aspect of the investigation,

The aims of this experiment were to investigate:

1. The relationship between plant species diversity and carbon concentration
2. The relationship between and functional group diversity and carbon sequestration
3. The sequestration of different carbon fractions of reactivity in relation to soil depth
4. Assess these over a 1-2.5 year timescale, as mimics a temporary ley
5. Seasonality in soil carbon fluctuations during the life cycle of the ley

3.2 Methods

3.2.1 WG1 establishment

Under controlled conditions, the pots were set up outside a greenhouse in the Walled Garden of the University of York (grid ref: SE 62506 50330). This experiment will be referred to as WG1.

The experiment was established in February 2020. The University of York Biology Workshops (Mark Bentley) prepared one-metre lengths of opaque, round, rigid PVC pipe, of 150mm internal diameter and 155mm external diameter (source: https://www.ducting-online.co.uk/pl150-rd-round-rigid-pipe-c2x14449876). The pots were created with such height to allow for deep-rooting species, and all species were planted in long pots alike to control for the effect of short pots restricting roots or reducing an insulation effect from the base. The long pots were drilled with holes of 20mm diameter, with the centre of the holes at 100mm, 300mm, 600mm and 900mm from the top of the pot. Using high strength adhesive, plant saucers of 165mm diameter (source: https://warboyshardware.co.uk/grow-saucer-terracotta-165cm-p-1022.html) were attached to the base of the pots.

Two tons of multi-purpose topsoil soil was sourced from East Riding Horticulture Ltd. Several options for filling the long pots were considered, such as topsoil with additives such as fertilisers; layering soil with sand to reduce nutrient load and create obvious stratification in the substrate to observe root growth; and mixing topsoil with sand in a cement mixer to create a completely uniform soil texture and reduced nutrient load (Redeker and Cicerone, 2004). However, with issues relating to time and cost of sourcing substrate and machinery, topsoil was used as a uniform but nutrient-high substrate, with any issues relating to texture and nutrient load unlikely to cause significant inconvenience or disruption to the experiment compared with the cost of mixing two tons of substrate by hand. The factor of water was also not controlled for, the only consideration being a lack of either flooding or drought. Plants were to receive water when it was required, usually relying on rainfall, given the application of the experiment to agricultural grasslands which would be established *in situ* where supplementary watering is unlikely in the UK. No additives or fertilisers were to be used, due also to the application to farmland, whereby grasslands are generally established on land with a legacy effect of fertilisers with either reduced (50kg/ha direct N application) or nil direct application but reliant on animal excreta (AHDB, 2022); in addition, the soil used for this initial experiment was by necessity of a higher organic matter content than average or agricultural land and this would contribute towards mimicking a fertiliser legacy effect or low input system. The plants would be allowed to grow, replicating a situation without grazing or a hay cut whereby in the field the main purpose for this would be an annual cut to maintain sward heterogeneity which is here maintained by plant separation in pots, for a minimal interference management system as is frequently observed in stewardship scheme sponsored grasslands (Natural England, 2015).

The experiment was established with one species per pot to observe the individual effect of the species on the soil, but with several individual crowns per pot to maximise green coverage, in order to limit C flux from bare soil. Five replicates of each species were planted for statistical power, but this number was limited by the sampling and processing time, with four samples to be taken from each pot every three months and the processing being time-sensitive due to the degradation of some organic acids, nutrients and gases after sampling. The diversity of species was also limited by this factor, and so were restricted to six species which are either compulsory or extremely common in all DEFRA-approved herbal ley seed mixes, representing grass, herb and legume species. The species were also chosen to represent different rooting morphologies, from shallow fibrous rooters through mid-depth down to tap rooting species.

The species selected were timothy grass (*Phleum pratense*), ribwort plantain (*Plantago lanceolata*), dandelion (*Taraxacum officianale*), chicory (*Cichorium intybus*), white clover (*Trifolium repens*), and yarrow (*Achillea millefolium*).

Timothy grass was selected as a common grass in DEFRA seed mixes. A grass was required as all grassland and herbal leys include at least one grass species. I chose to study one, as most grasses maintain a similar shallow fibrous rooting system broadly falling in the 10-30 cm depth range, and I intended to exploit future opportunities to study the effects of a greater variety of individual grass species *in situ* at the Halstead experiment (Chapter 5)*;* therefore the WG1 experiment could be focussed on herbs and legumes. The most common grass in DEFRA seed mixes is perennial ryegrass

(*Lolium perenne*) due to its hardiness, drought resistance, high calorific content and tolerance of high nutrient loads (T. Cook, 2022; Hannaway et al., 1999). However, over a period of 2-3 years, timothy gives a similar yield to perennial ryegrass, (Maunsell & Scott, 1996) while livestock grazed on a mixed grass sward including timothy have higher weights at slaughter (Grace et al., 2019). Timothy is also a less ferocious competitor, allowing greater plant diversity to be maintained in timothy-dominant meadows (Becker et al., 2020) which is also good for livestock weight gain (Grace et al., 2019), as ryegrass is likely to outcompete any co-existing herbs in both *in situ* planting and in future multi-species experiments (Becker et al., 2020). Ryegrass is also a better-researched plant and its effects on soil and soil carbon are already better understood (De Neergaard & Gorissen, 2004; Domanski et al., 2001; Meharg & Killham, 1989). For the reasons of deepening our knowledge on timothy, of extending its possible commercial use as a less competitive but equally nutritious grass for livestock which allows a greater variety of herbs and legumes to co-exist, and its presence already in many herb and grass seed mixes, I chose to study the effects of timothy on the soil over ryegrass.

Ribwort plantain is hardy perennial plant ubiquitous in agricultural grassland leys, surviving well in disturbed land including areas where livestock trample (Pol et al., 2021). This perennial is utilised for its high concentration of micronutrients for healthy livestock grazing (Harrington et al., 2006). Owing to its mid to deep rooting system of combined tap and shallow fibrous roots, ribwort plantain is a bridge species between the shallow and tap-rooting species to observe the effects mid-range roots may have on the concentration and depth of SOC.

Dandelion is a tap-rooting perennial, reaching depths of up to a metre (Mahr, 2022), common as a low-yielding but persistent and nutritious livestock forage within grasslands (Garden Organic, 2022). Covering a range of rooting depths was important to the study to establish whether there was a link between the depths of the roots (and thereby the porosity and influence of that on water content and the percolation of nutrients, and most of all the penetration of the root exudates), and the depths to which C was altered compared to the baseline.

Chicory is a deep-rooting woody perennial herb, representing another example of a tap-rooting species. Frequently grown as additional, micronutrient- and calorie-rich forage for livestock, and also for its parasite-suppressant properties (Athanasiadou et al., 2007), chicory is a common and beneficial addition to grasslands and leys which are used either for direct grazing or hay harvest. Therefore it was judged a suitable and important species to identify whether it holds other, carbon-related, benefits.

White clover represents the legume category, with at least one legume mandatory in all grassland and herbal ley seed mixes under farmland stewardship schemes. The rooting morphology of white clover also aligns with the categories under this experiment, with a tap rooting system which reaches shallower depths than dandelion but maintains a network at around 40-60cm deep.

Yarrow is also common in the NE seed mixes, included in wildflower, herb, legume, nectar, meadow and fallow seed mixes (FarmSeeds, 2022). It is used as livestock forage with its micronutrient content important for livestock health, essential oil production, and antioxidant properties (Bayat et al., 2021; Chizzola et al., 2003). Given its tolerance of wet and dry soil, variable elevations and nutrient concentrations, and disturbed ground, it is ubiquitous in farmland (and other areas such as parks, meadows, road verges, coasts) (Bourdot, 1980). It has a rhizomatous growth pattern, and shallow roots at around 20cm deep (Bourdot, 1980).

That all the species selected were perennial was an important factor for several reasons: this allowed the experiment to be maintained for at least a year (up to two), to allow for seasonal variation; it allowed for some natural tissue die-off, including that in the roots, over winter, with the following impacts of the natural regeneration cycle on the soil then observed; and it mimicked a more hands-off approach that might be observed in the field, whereby areas planted with grassland, herbal leys, or pollen or nectar resources are left uncontrolled over the time for which they are funded and left to germinate, flower again or die, which leads to natural out-competition of certain species over the funding period.

Cutting, mimicking mowing or grazing, was not undertaken in order to observe more natural die-off of the tissues, and removing organic matter from the soil would have reduced the returning nutrients.

The plants were sourced from a controlled allotment without inputs. Young crowns were carefully uprooted, the roots wiped off rather than washed to retain some inoculum of the soil bacteria, and the roots wrapped in damp kitchen towel within a plastic bag to maintain a moist environment. Enough plants were supplied that crowns of a similar size could be grouped together. Separated into 'small', 'medium' and 'large' groups, and depending on how many crowns of each species fell into each group, five replicates were formed of either one small and one large plant, or two medium plants, so that each replicate was of a similar size.

3.2.2 Sampling

Sampling was conducted with a small corer inserted into the pre-drilled holes, as described in Chapter 2. The soil of WG1 was sampled before anything was planted (baseline) and then plants were established in February 2020. Sampling was conducted in March 2020, June 2020, September 2020, January 2021, July 2021, and January 2022 to give a span over almost two years.

3.3.3 Laboratory processing

For this experiment, the laboratory processing included a full suite of C:N analysis and pyrolysis for C; all the basic parameters such as bulk density, water and pH; ion chromatography for nutrient content; and a partial dataset for VOAs as limited laboratory access due to the pandemic meant some samples were resident in the freezer for long enough to degrade the volatiles present in the soil and nullify the possibility of meaningful data.

3.3.4 Statistical analysis

Generalised linear models (GLMs) were run using free statistical software package R.

I considered GLM the best way to analyse my data as it could cope with parametric and non-parametric data, categorical and continuous variables, a high number of variables, and I was familiar with the organisation of the predictor and response variables. However, the analysis required some data manipulation. Due to the patchiness of VOA data, affected by covid-19 protocol which necessitated long periods in the freezer for many samples which degraded the acids, all VOA data was excluded from the GLMs. The final month (January 2022) was also missing total C, N and C:N ratio data, due to a combination of covid-19 delays, staff sickness due to covid-19, and finally a broken part in the analyser in September 2022. So January 2022 was excluded from the initial GLM.

I also removed all outliers from the data and any obviously incorrect data (>100% weight loss LOI values, for example) and forced R to read depth, month and species (as month and species were dummy coded) as categorical variables.

Another issue I did not manage to solve was R reading each individual phosphate value as a separate variable (despite no differences between how phosphate was input) and thereby refusing to run a model. I had to remove phosphate as a predictor variable for this reason.

Root data, gathered by destruction, was a smaller dataset. Multiple linear regression in R set total C % as the response variable against the predictors of root weight (g), depth (cm) and species. Time was taken out of the equation as roots can only be compared when they have a known measurement, i.e. at death, because the replicates were destroyed for the data.

3.4 Results

Total carbon, LOI at 20-250°C, LOI at 250-325°C, LOI at 325°C and total LOI were plotted against each predictor variable to check for linearity. These carbon and carbon-proxy values were used as the response variables in individual GLMs.

Results of C-GLM:

The initial GLM set total C (the value from the CN analyser) as the response variable and all others as predictors (month, species, depth, pH, water content, BD, nitrate, nitrite). The GLM was run with gaussian link. I will refer to this as the C-GLM.

- Deviance residuals approximately symmetrical either side of a median value of -0.33
- Water is a significant influence on C, $p = 0.017$
- BD is a significant influence on C, $p = 0.044$
- Total N is a highly significant influence on C, $p = 9.15 \times 10^{-11}$
- Nitrate is also significant, $p = 0.027$, although nitrite is not

Results of 250-GLM:

The second GLM I will refer to as 250-GLM. This places LOI at 20-250°C as the response, with the same predictor variables as C-GLM.

- Deviance residuals: 1Q = -2.589, median -0.713, 3Q 1.929
- The month the sample is taken is always significant, and significance increases with time
- March 2020 has a significant influence on LOI at 250°C, $p = 0.036$
- June 2020: $p = 0.021$
- September 2020: $p = 0.008$
- January 2021: $p = 0.001$
- July 2021: $p = 1.87 \times 10^{-14}$
- Yarrow, the most shallow-rooting herb, was also a significant driver in difference in LOI at 250°C, unlike the other species which were not significant. For yarrow, $p = 0.015$
- While there was no significant impact on the sample being collected at 30, 60 or 90 cm depth on LOI at 250°C, there was a difference at 10 cm depth, $p = 0.008$
- No other variables showed a significant result

Results of 325-GLM:

The third GLM, 325-GLM, placed LOI at 250-325°C as the response variable.

- Deviance residuals of 1Q = -1.639, median = -0.108, 3Q = 1.325

- 10 cm depth had a significant influence on LOI at 250-325°C, $p = 0.050$
- pH showed a significance of $p = 0.014$
- N showed significance of $p = 0.001$

Results of 550-GLM:

550-GLM placed the LOI at 325-550°C as the response variable.

- The median deviance residual was close to 0, with a value of -0.111. 1Q was -1.348 and 3Q was 0.947
- The third sample month, September 2020, was significantly influential on the most recalcitrant fraction of carbon, $p = 0.004$, although this time there was no grading in the significance of the influence of month and no other months showed significance
- Control pots were also showing as significantly different to the pots containing plants, $p = 0.043$
- N was also a significant predictor of LOI at 550°C, $p = 1.64\text{x}10^{-5}$

Results of LOI-GLM:

LOI-GLM placed the total LOI as the response variable and the other measures as predictors, as before.

- Deviance residuals of 1Q = -3.530, median = -0.850, 3Q = 2.419
- Time was significant: the first sample month, March 2020, showed p = 0.013.
- By September 2020, p = <0.001
- By January 2021, p = 4.9x10-5
- In July 2021, p = 0.007
- Yarrow was again a significant influence on carbon in the form of total LOI, p = 0.017
- Soil depth of 10 cm was a good predictor of total LOI, p = 0.002
- N was also significant, p = 0.001

Mean root weight (g) for each species over each depth interval, with standard error, is displayed in Table 3.1. ANOVA was run on a dataset which compared the root weight (g) and C (%) for the same pot, species and depth, although for C, a lot of 0-10 cm values were missing because of soil sinkage. This causes a problem in analysis because sinkage may not be evenly distributed throughout the pot and simply subtracting the distance in sinkage from the surface from all soil depth measurements does not incorporate this fact. Also, some pots sank up to 18 cm from the surface but obviously cores are still taken from the 90 cm pre-drilled holes so subtracting to make that 108 cm sampling depth does not work. This is acknowledged as an issue in the data analysis but at this stage, the sample depths of the C values and the rooting depths are used as 'true' values (i.e. accepted for the purpose of analysis that they are accurate) and compared to each other in 0-10 cm depth intervals. Because of a lack of root data below the 70+ cm line in each pot, root data are matched with soil cores of the shallowest depth measurement, i.e. 0-10 cm root data is aligned with 10 cm soil cores, 30 cm soil cores are matched with 20-30 cm root data and so on, although this means some data from 0-10 cm roots have no accompanying 10 cm soil core to take C data from. Where this occurred, a mean of the C content from other soil cores of the same depth and species was used to fill in the gaps. Additionally, because of the sinkage, this would mean losing 90 cm cores for deep C definition. Therefore the 70+ root data category was aligned with the deepest soil core of 90 cm depth as the last root portion of the core contained the 90 cm corer hole.

Because January 2022, the final sample month for WG1 after two years of growth, has an incomplete dataset due to problems with the C:N analyser, any missing C values for specific pots and depths were also filled in using mean C values from pots with the same depth and species to facilitate analysis. Only timothy had no 10 cm cores in the final sampling. Therefore timothy has no 0-10 cm root vs 10 cm depth C values included in the ANOVA.

Table 3.1 Mean root weight (g) over depth intervals

Species	Depth	Mean root weight (g)	SE
Chicory	0-10	11.69	4.87
Dandelion	0-10	11.90	0.53
RC	0-10	0.00	0.00
RP	0-10	3.62	1.64
Timothy	0-10	7.16	4.35
Yarrow	0-10	1.65	0.32
Chicory	10-20	2.97	1.76
Dandelion	10-20	8.28	2.30
RC	10-20	0.01	0.01
RP	10-20	1.99	0.59
Timothy	10-20	3.91	1.38
Yarrow	10-20	1.34	0.09
Chicory	20-30	1.91	0.62
Dandelion	20-30	7.13	1.88
RC	20-30	0.03	0.03
RP	20-30	1.51	0.65
Timothy	20-30	2.90	0.99
Yarrow	20-30	1.38	0.01
Chicory	30-40	1.85	0.46
Dandelion	30-40	2.98	1.59
RC	30-40	0.21	0.11
RP	30-40	1.48	0.75
Timothy	30-40	2.31	0.63
Yarrow	30-40	1.73	0.28
Chicory	40-50	1.69	0.34
Dandelion	40-50	4.00	0.71
RC	40-50	0.22	0.11
RP	40-50	1.14	0.57
Timothy	40-50	2.13	0.58
Yarrow	40-50	1.80	0.20
Chicory	50-60	1.25	0.66
Dandelion	50-60	2.87	0.67
RC	50-60	0.22	0.12

RP	50-60	1.33	0.19
Timothy	50-60	1.89	0.31
Yarrow	50-60	1.81	0.20
Chicory	60-70	2.37	0.66
Dandelion	60-70	2.52	0.33
RC	60-70	0.48	0.25
RP	60-70	1.01	0.40
Timothy	60-70	1.89	0.10
Yarrow	60-70	1.70	0.15
Chicory	70+	1.00	0.48
Dandelion	70+	2.05	0.26
RC	70+	0.10	0.05
RP	70+	1.24	0.40
Timothy	70+	1.39	0.12
Yarrow	70+	1.44	0.10

Multiple linear regression tested the predictors of root weight (g), sample depth (cm), and species against the response variable total C %, to observe the relationship between the predictors and between predictor and response. No significant variables were observed.

Figure 3.1 shows the relationship of root biomass (g) to the depth interval of the long pot from which the root portion was collected. Means of the three replicates destroyed for their roots are shown with standard errors. There are patterns in species, for example red clover consistently has the least weight and dandelion the most across most or all depth intervals. Chicory only has high root weight in the first interval, and yarrow and ribwort plantain root biomass are fairly consistent across all depth intervals.

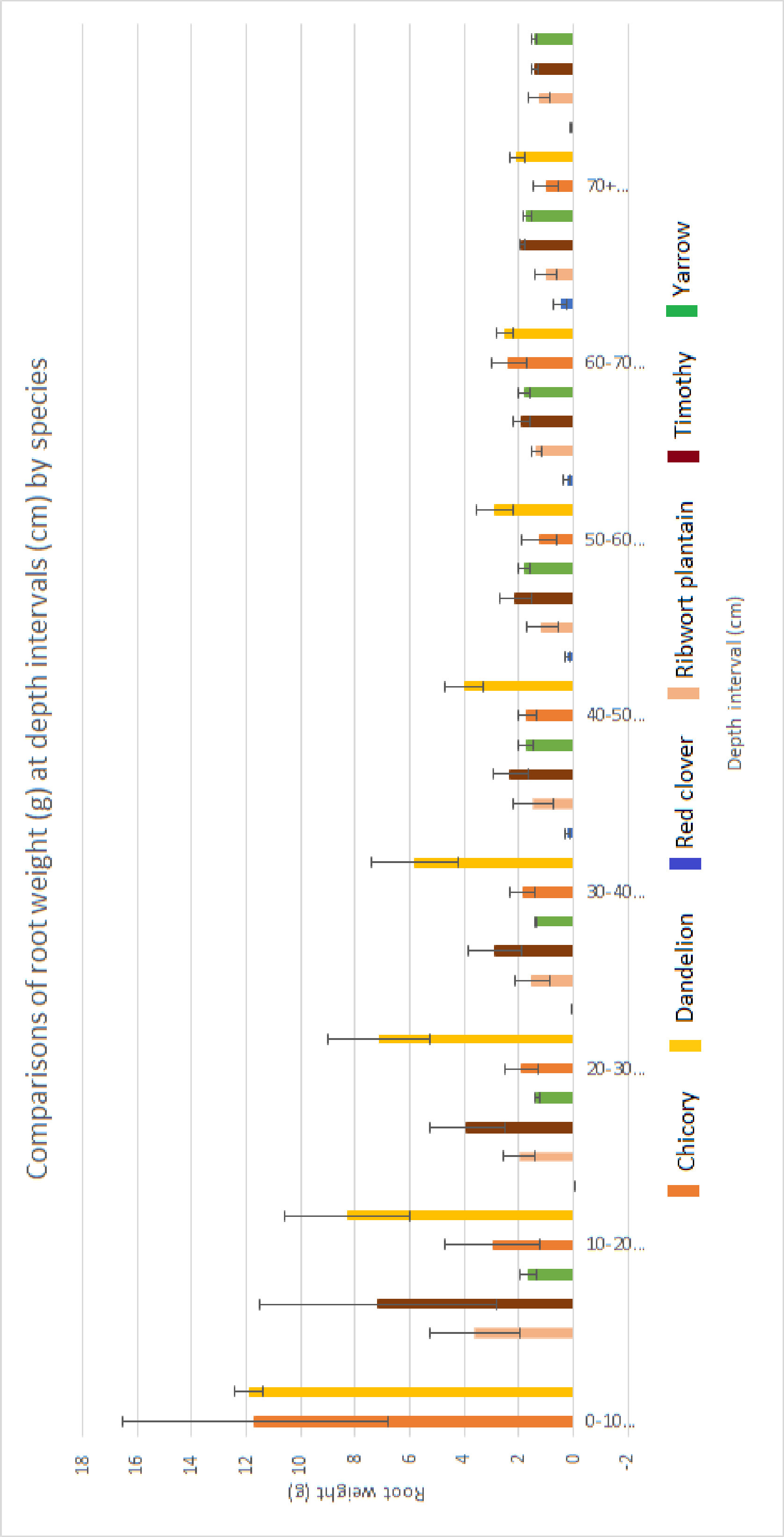


Figure 3.1 Species comparisons of root biomass across depth intervals

3.5 Discussion

3.5.1 Soil sampling limitations

There was a textural difference between the soil of WG1 and the soil of WG2. The soil of WG1 was sandier and lighter, despite having a higher C content, which is generally linked to darker soils and influenced by the presence of OM. This made it more difficult to filter the soil of WG1 for roots than the soil of WG2. Soil texture is determined by grain size which is influenced by particle scale of the parent material. Particle size is a factor in the SOC storage potential of the soil, as increased clay content increases storage capacity, but SOC stabilisation is also dependent on mineralogy (Bhattacharya et al., 2016; Kögel-Knabner et al., 2008; O'Rourke et al., 2015) and respiration from SOM decomposition by the microbial community (Xu & Shang, 2016). In terms of mineralogy, the base material influences aggregate stability, seal formation, runoff and soil loss (Lado & Ben-Hur, 2004). Grain size and mineralogy are also chemically linked: up to 65% of the organic C in mineral topsoils occurs in young particulate matter that is loosely or unconnected with soil minerals and can be rapidly reacted (Kaiser et al., 2002). Therefore it may be the case that, in this C-rich but light, sandy soil, that mineralogy is playing a greater role than clay content for holding C. Probably playing a more influential role are microbial respiration and SOM decomposition, as microbes can be active in any soil, but this soil was also very moist and in a temperate climate which creates hospitable conditions for microbial growth. Run-off and soil loss would be less related to mineralogy in a pot with a raised lip and no base drainage, as in WG1, but the mineralogy may still influence aggregate stability (which was low). It seems appropriate to surmise that the soil may have been C-rich because OM was mixed into a sandy soil and given little time to rot down and form a more natural soil structure, as it was bought from a horticulture firm.

Another result to treat carefully is BD, which may not have been particularly accurate or representative of the whole pot. This is because soil samples had to be taken through pre-drilled holes in the pots. This means the same soil was probed each time, possibly compacting it with the corer, potentially creating a hollow which would fill with crumbling soil, and creating a horizontal tunnel over time. Creating a physical space in the sample pots of WG1 may influence rooting depth and pattern, and there are ensuing changes to variables including water, C and N. BD directly affects microbial access to air and water with which to synthesise, respire and grow, and the microbial community is instrumental in the formation of C- and N-containing compounds which are stabilised in the soil (Sayer *et al.*, 2017). BD also affects water content and nutrient availability by way of affecting the permeability of the soil to water, roots and air (Lal and Kimble, 2000). This is not to mention potential for introducing bacteria, micro and macro invertebrates, and certainly oxygen into the soil directly via the pre-drilled holes as samples were taken. Introducing novel microorganisms may affect availability of C, N and other nutrients, as they synthesise or degrade compounds containing these elements (Sayer *et al.*, 2017). By measuring the outcome, I have potentially changed the outcome: BD itself may be inaccurate due to compaction or crumbling; roots may take a different path; and C and N are secondarily influenced by the introduction of space, air and potentially bacteria or other organisms.

A variable which was not measured was plant growth, although photos were taken. A comparison is shown below in Fig. 3.2 of WG1 over time. It was noted that the plants of WG1 grew far more aggressively than those of WG2, even those of WG2 which were single-species pots. This will be the result of a higher nutrient load, particularly C, already present in the soil. This has several implications: it is harder to observe patterns in C fluctuation in a soil already C-rich; the plants would likely have a different life strategy in a soil which is nutrient abundant compared to an in-situ low-C agricultural field, where they absorb as much as possible from the soil for fast growth; in a low-C soil they would be forced to assimilate more C from the air than they had to in the soil of WG1. These observations indicate to me that the plants will not have acted to their full potential as funnels of C into the soil, as they simply didn't have to absorb as much from the air with a more readily available resource in the soil. Of course, the carbon outcome may be different if a real-life crop like this was then ploughed back into the soil, but even then, I believe the effort is likely to have a better pay-off in a C-depleted soil as losses also tend to be proportional to existing C concentration (Schulze and Freibauer, 2005). This experiment is therefore not particularly representative of the capabilities of plants to sequester carbon into the soil by way of dead tissues and root exudates.

The growth of the plants, considering their rooting patterns, is also intriguing. Chicory is touted as a deep-rooting potential solution to soil C loss to the atmosphere and helping to reabsorb atmospheric CO2 and store it through deep roots. However, in a C-rich soil, I have found evidence that chicory adapts its rooting strategy to maximise efficiency. Almost no chicory roots were found below 20 cm in WG1, despite ferocious aboveground growth. This may be linked to the high-C soil, as chicory didn't need a tap root to access the nutrients it needed. This was also reflected in the stability of the plants, which were noticeably droopy by July 2020 and required staking to prevent them drooping below the 'surface' line of the pot, which was 1m above ground, and consequently falling over or uprooting themselves. Again, the act of staking would introduce some interference to the soil in terms of bacteria and oxygen, but just as a one-off event, and this was preferable to losing the plant to premature death.

Figure 3.2. The WG1 experiment one month post-establishment (PE) in March 2020 (top left); three months PE in May 2020 (top right); six months PE in August 2020 (mid left); 12 months PE in February 2021 (mid right); and a side view after 18 months PE with the chicory in second flower, August 2021 (bottom left); and almost two years PE in January 2022 (bottom right).

3.5.2 C-GLM

C-GLM indicated that water was a good predictor of total C. The literature of arid grasslands indicates that increased water content in soil can help to stimulate the metabolisation of C compounds by bacteria (Churchman and Tate, 1986). However, other research has indicated that the presence of water can reduce the C in soil by dissolving carbonate (Entry, Sojka and Shewmaker, 2002). The soils of the UK are generally moist but not wet in the temperate maritime climate, and higher moisture has been found to reduce CO2 emissions from soil (Curtin *et al.*, 1998). This is possibly because the presence of water is key to a process which transfers labile carbon to more recalcitrant forms, as most CO2 emissions were similar to the amount by which labile fraction C decreased, suggesting labile OC is a major source of respired C (Curtin *et al.*, 1998). It may also not be a strict causal relationship, because moisture is also related to the mineralogy of soil parent material which influences soil texture and mineralogy, which in turn determine moisture retention among other properties (Eze, Palmer and Chapman, 2018). WG1 has a high OM content, which retains a lot of moisture, shown below in Fig. 3.3 with the moisture content concentrated around the 35% mark. This may indicate that soil C emissions were limited by water-driven processes which reduce C respiration and increase the transfer of labile carbon to recalcitrant.

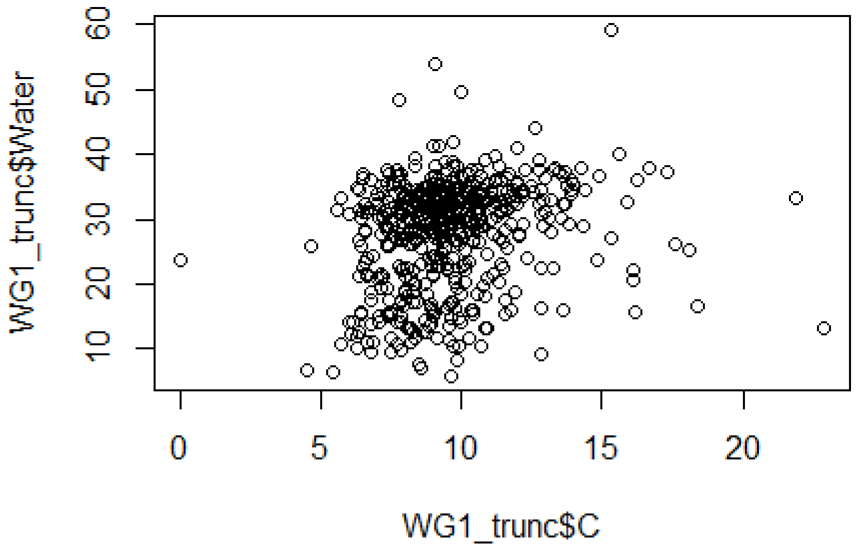


Figure 3.3 Total C content (%) plotted against total water content (%)

<u>Bulk density and C</u>

There is an established relationship in the literature between soil C and BD. In WG1, BD had an approximate correlation to water content (%), see Fig. 3.4. In high-C soils such as peat, the product of bulk density in g cm^{-3} and total C content (%) of the geobiological material are used to determine total C (Chambers, Beilman and Yu, 2010). The effect of BD on soil C and N is also greater at depths below 20 cm (Jurgensen *et al.*, 2017). Bulk density changes with land use, which is also influential on the C storage potential of soils, and with the plant species inhabiting it due to the pore creation and aggregation effects of their roots (Lal and Kimble, 2000). Related to water content discussed above, high moisture content in soil can increase BD (Wilson and Warren, 2015), although this may introduce a further element of variability into C analyses if soils are frequently wetting and drying which affects BD and feeds into microbial or root processes which seasonally fluctuate. BD also influences other properties affecting C stocks including water and nutrient holding capacity (Lal and Kimble, 2000). Bulk density in WG1 clustered around 1.00-1.25 and has a high C content of around 10% but up to 20% in some replicates – an increase of OC has been linked to a reduction in BD but by a mediating variable, in the case of this study, no tillage (Blanco-Canqui *et al.*, 2009). However, WG1 was also subject to no tillage so this may be relevant. Blanco-Canqui et al. (2009) also found the reverse pattern to be true, that SOC was a good predictor of BD, as SOC improves the capacity of the soil to resist compaction.

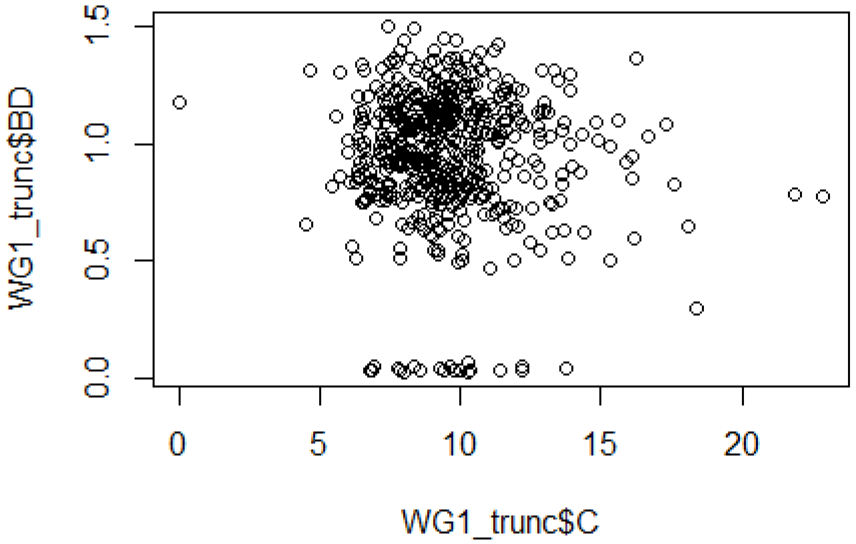


Figure 3.4 The correlation between BD and total C (%)

Total N and C

Linking to themes discussed in Chapter 4 regarding the role of N-fixing legumes in herbal leys, soil N has an established relationship to SOC. In situ in a diverse ley, grasses rely more on N synthesised by legumes, rather than N available in the soil (Dhamala *et al.*, 2017). Fertilisers have greatly increased biologically available N in the soil, which may help plants sequester more C in their tissues and partition resources to invest more in belowground rhizodeposition of biomass and exudates, as they overcome the usually limiting factor of N supply (Loubet *et al.*, 2011; Crowther *et al.*, 2019). It is likely in WG1 that all N was present in the soil in OM prior to the experiment being planted, because legumes were in their own pots with no crossover between legumes and grasses or herbs. There can be no link in WG1 between legume-derived active soil N and increased C in the pots of any other species than white clover. As white clover did not show as a significant predictor in terms of individual species effects on C, it is very unlikely that white clover with its N-fixing strategy is responsible for enough C fluctuation to drive N as a functional predictor of C. Therefore in WG1, the effect of N on C is coming from a source other than legumes.

Optimising N supply in low-C croplands can help to reduce C emissions and even reverse the effect into net C storage over six years, because C:N ratio stabilises around 9.5 (Ammann *et al.*, 2009). Mineral N fertilisation can reduce soil respiration rates, reducing the loss of CO_2 by microbial respiration (Lee and Jose, 2003). Mineral N fertilisation has been found to produce a lower microbial respiration rate than organic matter N in the form of cattle manure, possibly because of the added effect of manure to increase soil pH, which has an impact on respiring microbiota (Enwall *et al.*, 2007). No N inputs were added to WG1 over the course of two years, although the baseline soil would be likely to have had some inputs either artificially, or, more likely, by OM because of the high C content. High initial levels of N are likely to have prevented N acting as a limiting factor on plant growth, although accompanying high levels of C possibly made high investment in root biomass less necessary; therefore an initial drop in carbon was observed. Carbon fluctuated with seasonality (Fig. 3.5), supporting the theory that some rhizodeposition in terms of winter tissue die-off may have increased microbial respiration with a time lag.

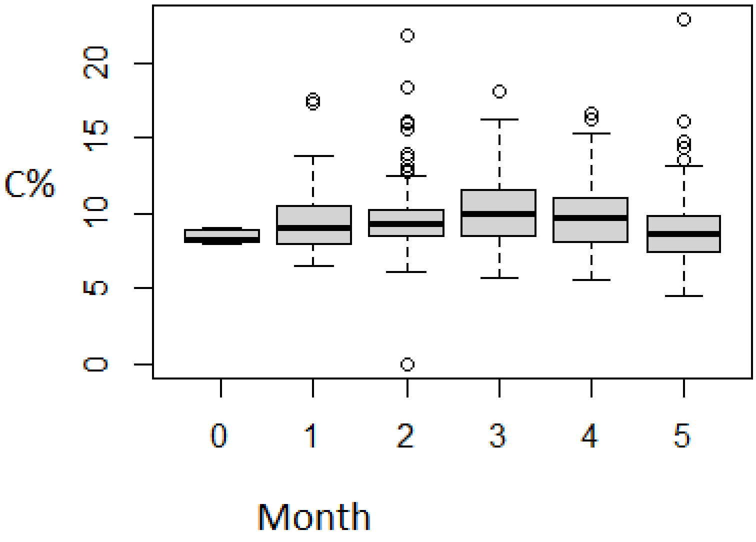


Figure 3.5 Total C (%) over all species and depths over time, with 'month' referring to sample months: 0 = baseline, 1 = March 2020, 2 = June 2020, 3 = September 2020, 4 = January 2021, 5 = July 2021

Nitrate, nitrate and C

Nitrate (NO_3^-) and nitrite (NO_2^-) are biologically active N compounds present in plants and water and produced by nitrifying bacteria in soil. Nitrate is highly soluble and biodegradable and also acts as an oxidising agent. Nitrites are primarily produced as byproducts as ammonia degrades to nitrate by the action of nitrifying bacteria. The result of nitrate as a significant predictor of C in WG1 suggests that degradation of N-compounds takes place quickly in a microbial community of abundant nitrifying bacteria. As nitrate is the more stable of the two, it is likely linked to an increased presence of C for reasons discussed above. Soil pH and OM content are key factors in nitrite decomposition to nitrate – neutral soils (like that in WG1) demonstrate a pattern of increasing nitrite fixing with increasing SOM (Nelson and Bremner, 1969). In this neutral, high OM soil, we would expect high nitrite decomposition. Soils high in C, like those in WG1, usually have active populations of denitrifying bacteria which may reduce nitrate leaching (Adelman and Tabidian, 1996). Nitrate deposition affects microbial carbon turnover in carbon-rich soils, as the active nitrate influences the mineralisation rate of labile forms of C (Currey *et al.*, 2010a).

3.5.3 250-GLM

Month and Lability

We expect to see the more labile fraction showing change over time, as most change is driven by the labile fraction in other studies (Rovira and Vallejo, 2000; Bernal *et al.*, 2016). In one 20-year study on converting forest to tea plantation, labile carbon losses were up to 71% compared to overall C loss average of 30% (Zhang *et al.*, 2022). Labile C is heavily microbially-derived (Zhang *et al.*, 2022) so this indicates an important role of microbial cycling in WG1. There may be a benefit in C being stored in labile forms in the face of climate warming, as decomposition of recalcitrant carbon is more sensitive to temperature change than labile carbon (Conant *et al.*, 2008). However, at the same time it is important to understand that WG1 indicates simply covering the soil with a crop is not enough to prevent labile C loss on soil with high SOC. Alongside the fact that most C losses are coming from the labile fraction, in WG1 and in the literature (Rovira and Vallejo, 2000; Jiang, Cao and Zhang, 2014; Liu *et al.*, 2020), we can state that simply cover cropping is not enough to prevent theses labile C losses, which are obviously a major contributor to soil C loss (Poeplau *et al.*, 2011). It is also evident from this that the functional groups tested in WG1 are not enough to prevent labile C loss over a period of two years or less in a C-rich soil. Time becomes a more accurate a predictor of labile C the longer the experiment has been established, which indicates both seasonal and long-term C changes in the soil with a distinct pattern. As illustrated in Fig. 3.5, the total C content fluctuates, with a slight increase followed by a decrease in C. This is likely to be an effect exacerbated by high initial C content, as there was already more C in the soil to lose.

Yarrow and Lability

Yarrow tends to have the most consistent root biomass at every depth interval (Fig. 3.1). From the literature and farming information, yarrow was expected to be the shallowest-rooting species tested in WG but chicory had less root across most depths than yarrow (Fig. 3.1). Timothy was also investigated as a shallow rooter but timothy also had consistent and deep roots, with roots found in both these ‘shallow’ rooting species at 70+ cm. Only dandelion had more root biomass at 70+ cm than yarrow, with timothy around the same weight. Soil C under yarrow was on average second only to ribwort plantain (Table 3.2). However, it is interesting that only yarrow is a good predictor of soil C in the most labile fraction. Yarrow reproduces both rhizomatously and from seed, and it does invest around 10% its dry weight tissue in rhizomes and roots belowground (Henskens, Field and Bourdôt, 1992), which may be expected to contribute to belowground C. Yarrow concentrates its carbohydrate into high concentrations of fructan in rhizomes and roots, but this does seasonally fluctuate with greater resource allocation to root growth in spring (Henskens, Field and Bourdôt, 1992). There is little to no research on the effect of yarrow specifically on soil C, so this is an interesting finding.

Table 3.2. Experiment species listed from highest average soil carbon to lowest (%), with accompanying mean root biomass per 10 cm interval (g)

Species	Mean root weight (g)	SE	Mean C (%)	SE
Ribwort plantain	1.924	0.488	9.898	0.567
Yarrow	1.570	0.098	9.659	0.343
Chicory	3.960	1.716	9.373	0.618
Red clover	0.087	0.039	9.357	0.345

Dandelion	5.990	1.262	9.251	0.562
Timothy	1.546	0.324	6.368	0.445

Ribwort plantain has the second highest mean root weight per 10 cm interval and the highest mean C (%), but also the third highest standard error for root biomass and second highest standard error for mean C content, meaning that there may not be a stable pattern for the model to identify a significant result. However, yarrow has the third highest mean root biomass (g) after dandelion and ribwort, and the second highest C, with the second-lowest and lowest standard errors for root weight and C respectively. This indicates much more consistency which may enable the model to identify the significant prediction between the presence of yarrow and the soil C content.

Because of the relationship between new carbon and shallow depths (Qin *et al.*, 2021), it is not unexpected that deeper-rooting plants are not driving change in the most labile C fraction, as they expected to have greater influence at depth. In addition, there are unexpected deviations in rooting depth from the expected morphologies in both shallow and deep rooting plants in WG1 which may be disrupting results. This is explored more below in section 5.3.7. The comparisons of root biomasses and C indicate that there are other drivers of labile C besides root biomass.

Lability at 10 cm

It is a key finding that the difference in SOC in WG1 is mostly driven in the top 10 cm soil, and that this labile fraction is significant. This tallies with the literature (X. Wang *et al.*, 2013; Crowther *et al.*, 2019; Sahoo *et al.*, 2019; Yang *et al.*, 2019). The possibility has been referenced in this thesis that soil at plough depth (10-30 cm) is over-represented in the literature because of its agricultural relevance, and there is a lack of information for soil below the O-A horizon which has implications for the application of current research to deeper carbon stores, and also inflicts bias on the available data. However, in WG1 when soil is tested even to 90 cm depths, the 10 cm range is still the most powerful in terms of driving C flux. In untilled soil, C accumulation tends to take place in the top 5 cm of soil (Quincke *et al.*, 2007) and total and labile C tend to decline with depth (Yang *et al.*, 2009; Qin *et al.*, 2021). Formation of soil C is positively correlated with labile C input rates (Bradford, Fierer and Reynolds, 2008), and WG1 was initially a high-input soil (although no inputs were applied over the two years of mesocosm life). This is likely to contribute to formation of labile C in WG1, rather than recalcitrant C, because labile C is driving the C changes in the experiment and there is a link between labile C and shallow range and formation time. Newer C tends to be limited to the surface layer of 0-10 cm (Qin *et al.*, 2021) and older carbon tends to be older (up to hundreds of thousands of years in soil of >1 m depth) and more recalcitrant (Bernal *et al.*, 2016). Microbes are significant drivers of soil C assimilation, there have been indications that microbially-derived C is the source of 64% C in the top 0-20 cm soil (Kramer and Gleixner, 2008). While the finding that most change in SOC stock is taking place in the top 10 cm soil is not ideal in terms of ley management for long-term SOC storage, it is important to know that over two years the C stored is likely to be subject to rapid change. It is also an indication that removing this top 10 cm from the surface and burying it could reduce the turnover of new C (Schiedung *et al.*, 2019).

It is interesting that no other depths are showing as significant predictors of soil C, not even in a negative relationship. While microbially-derived C is the source of 64% the C in the top 0-20 cm soil, it also contributes 34% of the C at 40-60 cm depths (Kramer and Gleixner, 2008). Total C, however, may be lower, which may explain why even active microbial communities are not driving a depth differential in C.

3.5.4 325-GLM

Mid-lability and 10 cm depth

Like the most labile fraction of C which burns off at 250°C, there is a relationship between the semi-labile carbon burning off at 325°C and depth. Only 10 cm samples drove the change, however, this is again not ideal as it indicates a low likelihood of ley treatments creating a lasting change towards deeper and more recalcitrant soil C. It is nevertheless an important finding, as the subsidisation of ley treatment for the purpose of C sequestration is likely not a good use of funding directed solely towards soil carbon (other benefits, such as biodiversity enhancement, notwithstanding). A slightly more recalcitrant form of C might have been expected to accompany more of a depth effect, which is not happening in WG1. This could be because of the speed of turnover if microbially-derived C is driving any SOC changes, as microbial C has a much faster turnover time than bulk SOM (Kramer and Gleixner, 2008).

Mid-lability and pH

Humic acids are a likely source of pH fluctuations, as these form through invertebrate and microbial decay of plant and animal tissues through humification. As the soil of WG1 was initially high in OM, this could be a contributor to pH differences. There is a relationship between pH and time (Fig. 3.6) as the pH fluctuates seasonally. In the GLM, pH is a significant predictor of C content in the semi-labile fraction. The relationship between pH and carbon fraction could derive from the breakdown of tissues into humus producing acids. The carbon produced by humification, the byproduct of which is humic acids, fulvic acids and humin, could be in a transitional phase from labile to recalcitrant carbon by partial mineralisation (Matus *et al.*, 2014; Bayat *et al.*, 2021). If labile carbon is very fresh, derived from recent OM inputs, humic acids could be a midway point between labile and recalcitrant C. This relationship to pH is not shown in labile or recalcitrant C so this indicates a relationship to a mid-range lability, or a mid-transition phase in carbon assimilation in the soil.

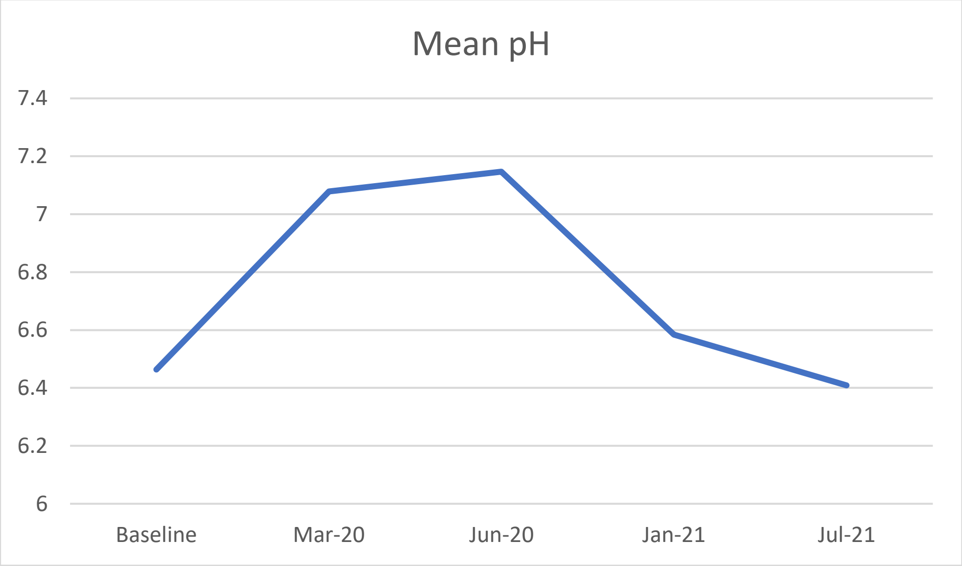


Figure 3.6 Fluctuations in pH over time with season

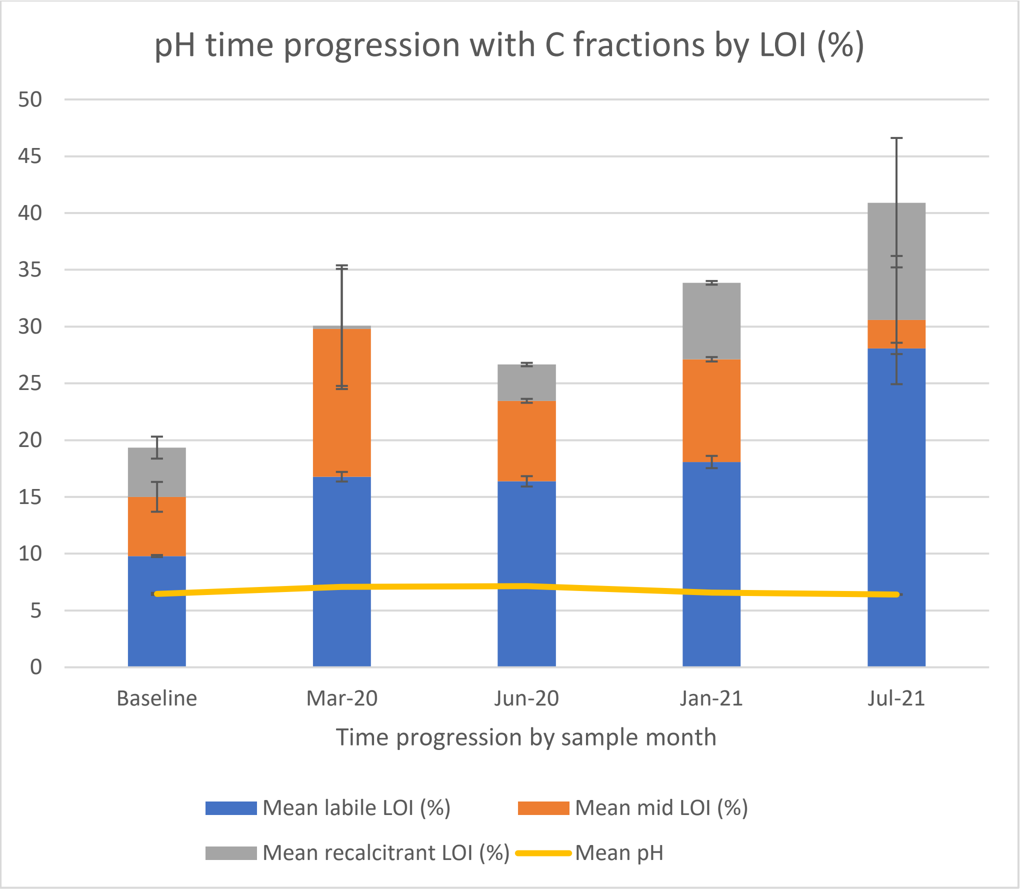


Figure 3.7 Fractions of C by LOI (%) and the relationship to pH

Soil acidification causes soil inorganic C loss and can affect SOC dynamics: decreasing soil pH decreases inorganic carbon but increases organic carbon in a soil with carbonate parent material, and this process is generally faster in shallower soils than in the subsoil (Shaofei and Wang, 2018). This could be another explanation of why only mid-lability C has a relationship to pH. Setting pH against carbon losses in fractions (Fig. 3.7) shows that the increase in weight LOI, indicating increased soil OM and specifically the largest percentage of mid-lability C in March 2020, correlates to an increase in pH from a mean of 6.45 in February 2020 to a mean of 7.1 in March. Mid-lability carbon could be in between the C reacting at lower temperatures which is dominated by organic carbon and the C reacting at the highest temperatures which is likely to have a slightly higher inorganic C content.

Nitrogen and mid-lability

Nitrogen and the 325°C fraction of C has a correlation, as does N and the recalcitrant C range, but *not* at total C, 250°C, or for total LOI. N would be expected to have a relationship with C regardless of fraction. It was anticipated that a link would be established across all C measures, perhaps even labile C in particular as this is where most of the change in C is over the course of WG1. There is a mediating factor of soil moisture which influences both mineral N and labile C (Oduor *et al.*, 2018), so the link may not be direct but a secondary effect of other soil characteristics, which have greater influence on the more bioactive C. Nitrogen can inhibit soil C decomposition from labile inputs, which offsets some of the drop in C formation observed when inputs of labile C increase (Bradford, Fierer and Reynolds, 2008). The addition of N has also been shown to mitigate some of the effects of residue removal in crop fields, stimulating microbial activity after residue removal usually causes a drop in activity (Stewart *et al.*, 2018) and microbial activity is an important driver of soil C (Fitter *et al.*, 2005; Schaufler *et al.*, 2010; Lal, 2013).

Mid-lability and limited drivers

Only three variables – depth at 10 cm, pH, and N – are predictors of mid-lability C. No other variables are effective predictors of mid-lability C. One possible explanation for this is that the temperature interval of 75°C is smaller than the 230°C to burn off labile C and the 175°C interval between mid and recalcitrant C. This could simply mean a smaller proportion of C is burned off at this interval than the labile or recalcitrant temperature intervals, providing a smaller dataset which gives less reliable and accurate conclusions. Another reason is that mid-lability C is less different from labile and recalcitrant C than labile/recalcitrant are from each other at opposite ends of the spectrum.

3.5.5 550-GLM

Recalcitrance and time

In the most recalcitrant fraction, only the month of September 2020 showed as a significant predictor of C. There is no grading in the significance of month with recalcitrant carbon, unlike labile. This could mean it becomes more stable over time after an initial fluctuation. There are small seasonal changes in soil C due to seasonal changes in rhizodeposition of roots and dead tissues, falling leaves, and decomposition of these, or investment in roots and new tissues which require C inputs (Wuest, 2014). Annual C variation is up to 16% the mean over a three-year period in the top 20 cm of soil, with 2-8% a regular seasonal pattern (Wuest, 2014). However, the Wuest (2014) study only examined down to 20 cm depth, and there is a link between deeper subsoil and more recalcitrant C in the literature (Soussana *et al.*, 2004; Shi *et al.*, 2013). Because recalcitrant C is less reactive, it would be expected to be less subject to seasonal fluctuations, which may explain why these patterns are evident in the 250-GLM and not in the 550-GLM.

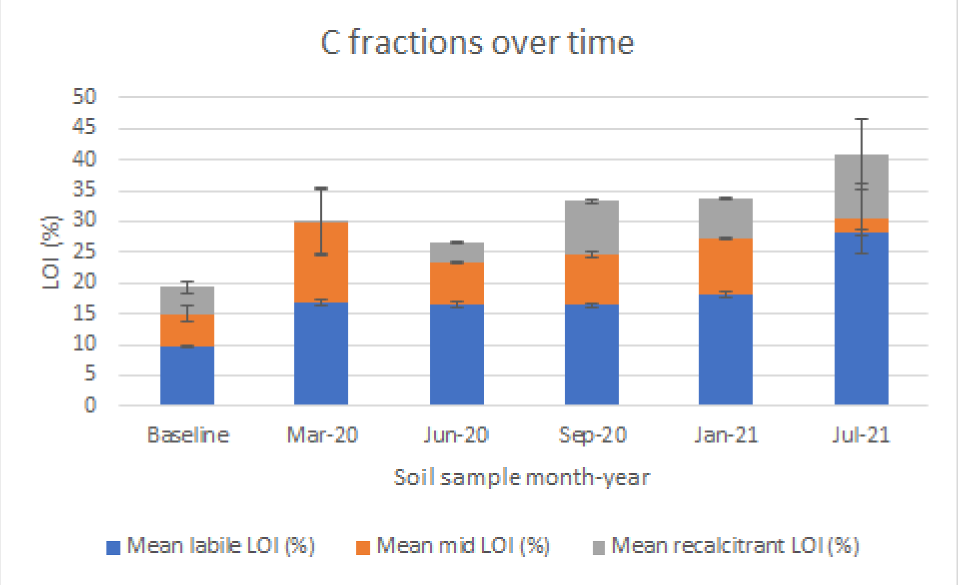


Figure 3.8 Carbon in fractions of lability by LOI (%) over time

The origin of the September fluctuation could be that an initial drop in SOC following the spring/summer season of vigorous growth (Fig. 3.8) meant that even recalcitrant fractions of C were accessed by plants to fuel biomass accumulation. However, this would contradict the theory that WG1 chicory plants only developed short and stumpy roots (see Table 3.2 for root biomass data) rather than long tap-roots because there was an abundance of C already easily accessible. The mechanisms by which labile C is transformed to recalcitrant are still not well understood (Fontaine *et al.*, 2007; Von Lützow *et al.*, 2008; Barré *et al.*, 2010). Recalcitrant pools of C even in subsoils are affected by land-use change, and cultivation decreases SOC stability (Liu *et al.*, 2020), so even if an increase in recalcitrant C caused a pulse after the first summer, it could be cycled back into the plant community rather than stored in lower soil horizons.

Recalcitrance and bare earth

Long term bare fallow experiments have been used to examine the effect of leaving soil bare. In comparison to the species pots, control pots were entirely open to the elements with no plants allowed to take root throughout the WG1 experiment. A cover crop has been consistently proved to increase soil C compared to bare ground (Hu *et al.*, 1997; Poeplau and Don, 2015; Jian *et al.*, 2020). While plants inherently provide soil inputs via exudates, roots and other dead tissues, inputs to bare soil are considered negligible in comparison, although they receive small amounts from atmospheric or wind deposition and occasional weeds (Barré *et al.*, 2010). Bare fallow plots can track the decay of soil C, in particular stable C because of a lack of inputs (Barré *et al.*, 2010). Total soil C always reveals a consistent decline over periods of up to 60 years bare, but the trend is illustrated over periods as short as the first year, and even more pronounced in soils with high initial C content (around 30%) compared to those with low starting soil C concentrations (around 15%) (Barré *et al.*, 2010). The processes behind this have already been discussed in Chapter 1, including the lack of roots to stabilise soil, leaving it vulnerable to erosion (Reubens *et al.*, 2007); this is supported by evidence of a much greater drop in the surface level of the control WG1 pots compared to those which contained plants. Other reasons include a lack of OM inputs, which prevent C cycling (Sainju *et al.*, 2007); a lack of support for microbial dynamics which influence nutrient availability and C (Hu *et al.*, 1997); and decreasing mineralisable C inputs (Jian *et al.*, 2020).

Given that only the absence of plants was a good predictor of recalcitrant C outcomes ($p = 0.043$), the presence of plants must be significantly different but the differences between the plants of WG1 are not enough to drive an individual species-related effect.

Recalcitrance and nitrogen

N is a significant predictor of recalcitrant C, as well as mid-lability C. It is interesting that it isn't a predictor of labile carbon, as this is the fraction we expect most change to be driven by (Rovira and Vallejo, 2002; Currey *et al.*, 2010b; Liu *et al.*, 2020). After an initial drop between the baseline data and March 2020, the recalcitrant fraction slowly builds until a slight drop in winter 2020-21 and then increases again (Fig. 3.8). Long term C storage is driven by this fraction of C which burns off at 325-550°C (Jiang, Cao and Zhang, 2014). Increasing soil N increases the labile carbon but decreases recalcitrant C, while also acidifying soil which can reduce bacterial biomass (Jiang, Cao and Zhang, 2014). Increasing SOC is usually limited by reduced N availability (Deng *et al.*, 2014), and there were no inputs into this soil post-establishment of WG1. It could, therefore, be that N is limiting the assimilation of C into subsoil recalcitrant compounds (Currey *et al.*, 2010b). There could be other mediating factors too, including microbial activity and the parameters which influence this including moisture and pH (Fitter *et al.*, 2005; Schaufler *et al.*, 2010; Lal, 2013; Oduor *et al.*, 2018).

Nitrogen present in humic and fulvic acid can cause pH fluctuations, and pH can also be an influencing factor on microbial activity (Qi *et al.*, 2018; Irfan *et al.*, 2019). These acids form through humification of OM. As the soil of WG1 was initially high in OM, this could be a contributor N differences both seasonally, as the pH changes indicate, and over time with OM breakdown (Fig.

3.6). A slight reduction in the scale of the drop towards the end of the experiment may indicate a slowing of humification and/or a transition away from fresh OM inputs contributing to acid production in the mid-lability fraction, towards a more recalcitrant C store, as indicated by an increase in the % C stored in the most recalcitrant fraction between over time (Fig. 3.8).

Recalcitrance and depth

No depths showed as good predictors of recalcitrant C content. This is not what was expected. In the literature, there is some indication that depth is linked to recalcitrant pools of C, either by the length of time they have been buried away from surface catalysts such as disturbance and air (Fontaine *et al.*, 2007; Shi *et al.*, 2013; Jiang, Cao and Zhang, 2014); because the proportion of alkyl C increases with increasing soil depth, and alkyl C enriches C structures and increases their resistance to decomposition (Lorenz *et al.*, 2007); because depth reduces O2 or water availability and affects the processes of C mineralisation which forms recalcitrant C (Rovira and Vallejo, 2002); or by leaching down of compounds which are harder to decompose and metabolise for plants and microbes which therefore limits their uptake into plant and microbial communities (Lehmann *et al.*, 2020). Soil organisms, including macroinvertebrates, are crucial in mediating C stability (Ameloot *et al.*, 2013; Lehmann *et al.*, 2020) and the macroinvertebrate community, while unmeasured, was anecdotally extremely poor in WG1. This result for recalcitrant C may indicate that the lack of this macroinvertebrate community is affecting normal soil processes which would usually cause a difference in C recalcitrance at depth. Because of relationships observed with pH, N, nitrates and nitrites, it would seem more likely to base this assumption on a lack of macroinvertebrate community in WG1 than a lack of an active microbial community. This is not a positive result in terms of sequestering unreactive C in situ in leys on degraded soil, if the soil fauna is not there to support C biodegradation (Ameloot *et al.*, 2013). It is also not a positive result in terms of the effectiveness of sequestering unreactive C in a short period of time, the lifetime of the average temporary ley, if the recovery of macroinvertebrate communities takes a longer time than 2-5 years. Recovery of macroinvertebrate populations in former arable fields to a level on a par with grasslands never cultivated can take 8-21 years (Wodika and Baer, 2015).

3.5.6 LOI-GLM

LOI and time

The time over which WG1 had been established was always a significant driver of LOI, but when split into fractions, only labile C showed as being significantly driven by time, with a close-significance result in the most recalcitrant fraction. This shows that it is the ends of the spectrum of recalcitrance – mostly the labile fraction, but some contribution from recalcitrant C where we also see building over time (Fig. 3.8) – which is driving change in C. This is supported by the literature where significant soil C change can take many years to observe (Kahlon, Lal and Ann-Varughese, 2013; De Moraes Sá *et al.*, 2014; Poeplau and Don, 2015; Han Weng *et al.*, 2017) and often initial fluctuations are driven by labile C (Curtin *et al.*, 1998; Bronick and Lal, 2005; Quincke *et al.*, 2007).

There is also a fluctuation in significance with time for total LOI, not the purely increasing significance over time observed in the labile fraction. This indicates a seasonal fluctuation in C (Fig. 3.8). This happens because of natural seasonal tissue die-off in winter, including root tissue and the dropping of leaves which are incorporated into the O horizon by microbial and invertebrate action (Bernal *et al.*, 2016; Bray *et al.*, 2020; Griffiths *et al.*, 2021). However, the patterns may not be as distinct in total LOI as they are in the most labile fraction, as most new C from the degradation of fresh plant derived organic material is incorporated into the soil as bioactive labile C (Jiang, Cao and

Zhang, 2014; Whitehead *et al.*, 2018). Therefore while this fraction may show the greatest response over time, fluctuations in more recalcitrant fractions influence the total LOI enough to make the pattern less distinct.

LOI and species

In terms of species-related influence, yarrow again acted as a strong predictor of total LOI, but no other species, or control pots, did so. That the pattern is observed in yarrow but not timothy when they have very similar root biomasses across all soil depths may indicate that either there are rooting differences significant enough to drive subsoil C changes; for example, similar biomasses do not take into account differences in morphology or root surface area, which may drive change by increasing the area from which exudates are released, nutrients incorporated, or how easily the tissue can be broken down by the microbial community. It could also be a different actor entirely apart from roots such as the lack of seasonal breakdown in yarrow subsoil tissue (Bourdot, 1980) or differences in exudates exerting some control over the abundance, diversity or function of the associated microbial community (Doornbos, Van Loon and Bakker, 2011; Huang *et al.*, 2014). The fact that yarrow is the only significant species driver in both the labile C and the total LOI again indicates that the majority of change in LOI is being driven by labile C change. The carbon in yarrow pots tended to be more consistent than other species, and held the second highest total C after ribwort plantain (Table 3.2). The physical characteristics of yarrow may, again, be influencing C storage as in the labile fraction with its 10% belowground investment including rhizomes (Henskens, Field and Bourdôt, 1992), which may be expected to contribute to belowground C, and as fresh OM would be expected to add to the labile C pool.

LOI and depth

A sample depth of 10 cm is a very significant predictor of total LOI. As discussed above, it seems that this is driven mainly by fluctuations in labile C (Fig. 3.8). That 10 cm is the only depth which shows a significant predicting effect links lability to shallowness. However, no distinct link between recalcitrance and depth has been established as neither recalcitrance or lower subsoil sampling depths have shown a significant predicting effect over a two-year time period.

It is also notable that two out of the three chicory replicates destroyed for their roots had almost no root below 20 cm depths, despite being included in the experiment for their well-known deep rooting characteristics, usually growing a tap root down to 1 m (Table 3.2). Chicory was included in the experiment for its deep roots, which indicate potential to influence soil processes at depths below the O-horizon which is where most C fluctuation is usually observed. The root that was present in the chicory plants was very thick, like a tap root, but very short; despite this, these short roots were provisioning plants which were over 1 m tall, with multiple stems, vigorous growth, and which flowered two or three times (chicory usually lives for two years). The chicory plants outlived other mid- and tap-rooters including ribwort plantain and dandelion, and given that they were alive into mid-2022, I think it is unlikely that microbial or soil fauna action would be able to degrade any existing tap roots in such a short space of time. The additional observation that the chicory plants almost toppled over and required additional support in the form of metal canes driven into the soil to prevent them drooping below ‘soil’ level (1 m above ground in the long pots) indicates a root too short to adequately support the plant. As an additional comment on supporting the chicory plants, driving in a cane will potentially have disturbed the soil and root, potentially temporarily increasing air exposure and introducing new bacteria into the pot, which could have caused disruption; however as a one-off event, this was preferable to uprooting due to the weight of the plants and potentially unstable root, which would cause premature plant death and remove them from the experiment. I don’t believe that the root tissues degrading to the extent they are undetectable in the long pots during destruction is a likely prospect, given the characteristic of chicory as having a thick ligneous tap root. I hypothesise a likelier explanation is that the vigorous growth and large diameter but short length of of tap root, causing instability of the chicory plants, is a result of the high C load in the soil. There would be no need for chicory to develop its usual tap root system given the plentiful nutrient availability, which requires less resource partition to root tissues for the same gain. As this topsoil was 10% C as a baseline average, and chicory can grow well in agricultural soils which can be as low as 2% C, it might be that up to 80% reduction in root investment could provide a similar level of nutrient availability to aboveground tissues. Root reductions of less than 80% in such high C soil could deliver higher growth rates than in agricultural soil even with less root, explaining the vigour of growth despite stunted roots. This leads on into the fact this experiment has its limits for drawing parallels with *in situ* plants, because the length of the root in the field would have other effects including increased susceptibility to wind damage and uprooting, potentially leading to an increase in soil erosion and soil C loss. However, as discussed above, leys including chicory and other deep-rooting plants are only desirable as a potential C-increasing measure on soils which are already degraded and low in C.

However, a slightly increased C content over time in chicory is observed, indicating that there are other actor/s besides rooting depth influencing C content, such as exudates which influence microbiota (Doornbos, Van Loon and Bakker, 2011) or associated fauna (Bray *et al.*, 2020). This could also be mediated by other factors which influence, or are influenced by, microbiota as the lead drivers of C change, including moisture which is influenced by roots changing soil porosity (Gelsomino and Azzellino, 2011; Cui *et al.*, 2019), or by pH which can be driven by changes in OM and humification processes (Kemmitt *et al.*, 2006; Currey *et al.*, 2010a; Bayat *et al.*, 2021).

3.5.7 Roots and depth influence

There was a weak positive correlation between root biomass (g) and soil C (%) (Fig. 3.9). Multiple linear regression showed no significant predictors when depth (cm), root weight (g) and species were tested against the response variable of total soil C (%). This could be because there was not enough time for roots to develop and senesce over two years to the point that the OM inputs could be assimilated into SOC stocks. This could be exacerbated by the fact that the soil was high in C initially, and nutrient enrichment meant plants could partition more resources into aboveground biomass production without investing heavily in root biomass to facilitate that. It is generally root biomass which contributes OM to the subsoil C stock via faunal and microbial biodegradation processes, and if roots were variable between species (as expected with the variation in root morphology) but shorter than usual due to a lack of C or N limitation, patterns between root biomass and C will be harder to pinpoint.

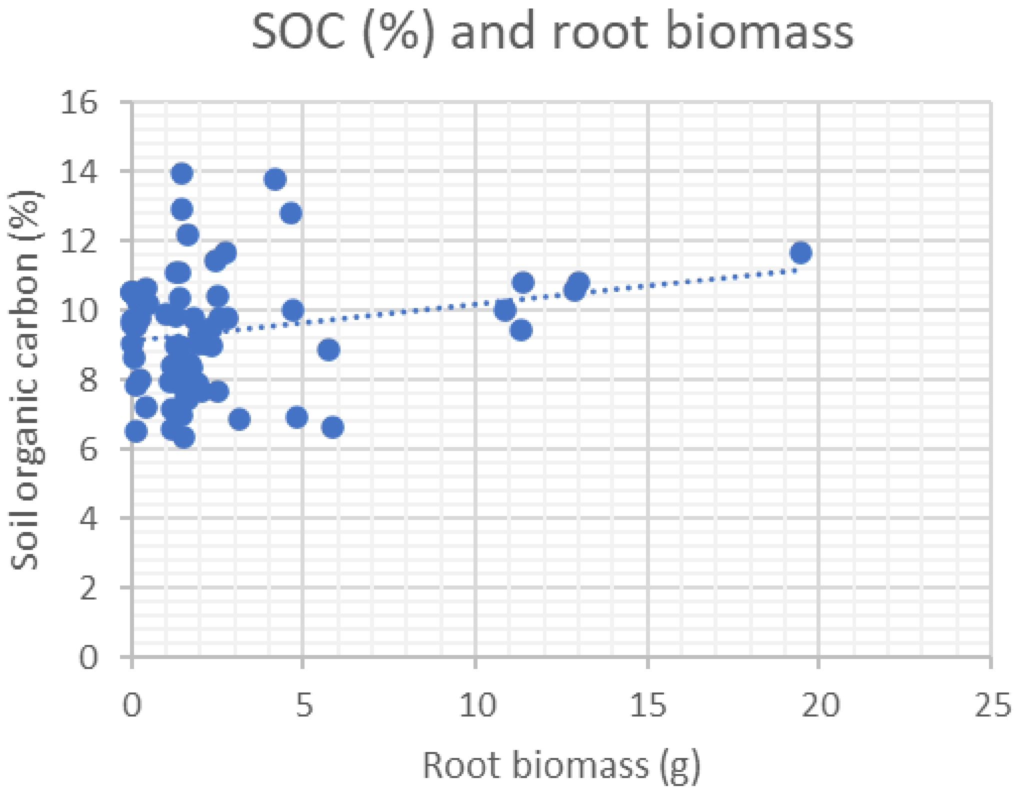


Figure 3.9 Comparing SOC (%) with root biomass (g)

Timothy also had an unusually low biomass:C ratio and red clover an unusually high ratio compared to the other species (Fig. 3.10). Ribwort plantain had the highest C overall but not the highest root biomass. Dandelion maintained its status as a deep rooter even in high C soil, but as discussed above, chicory did not and had very low root biomass below 20 cm whereas ‘shallow-rooting’ timothy and yarrow maintained some roots down to 70 cm and below (Table 3.2).

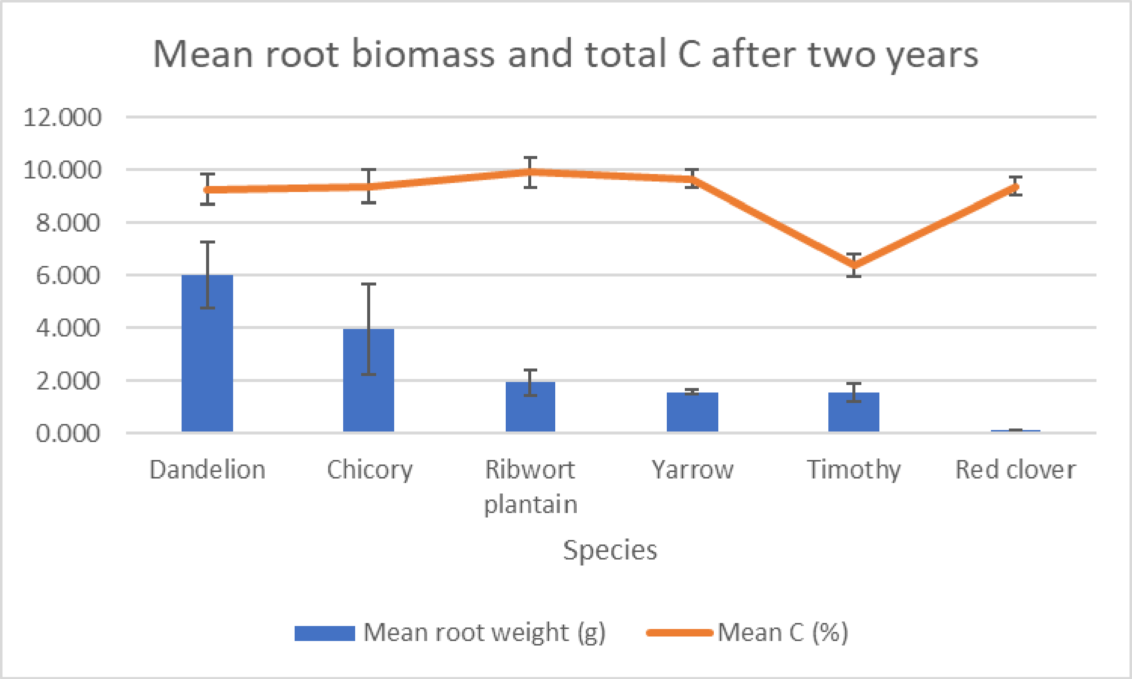


Figure 3.10 Mean root biomass (g) and total C (%) after two years

3.6 Conclusions

3.6.1 Soil parameters

Water is a good predictor of total C, which may be due to direct or indirect influences on soil C emissions such as high water retention in OM which has a higher initial C content and therefore more to lose; because the presence of fresh water increases the activity of some C-synthesising microbes; or because the presence of water pores correlates to the presence of O_2 pores which both provides a resource for aerobic bacteria and the oxygen for C to react with to form CO_2.

Soil stability is influenced by water holding capacity, related to OM content; by water run-off; and by drainage. While the OM content was high, improving water holding capacity, drainage in the WG1 mesocosms was poor. In combination, these factors may have had some influence on physical soil stability and thereby secondary effects on C and nutrient retention.

BD correlates to water content, and may be another (not statistically significant) contributor to C and nutrient retention through indirect effects. However, there are considerations to make regarding BD. The act of measuring BD affects its value. This also brings the possibility of indirect effects on BD-influenced variables, including rooting depth and structure, water movement, and O_2 supply. These BD-related factors may thereby cause tertiary effects on soil C and nutrients.

Mid-lability C was related to pH, likely influenced by the process of humification which produces acids and breaks down OM into C compounds.

There are indicators that WG1 had a healthy microbial community and the soil was always covered by plants (except in controls), and yet still lost C.

3.6.2 Nitrogen

N has an expected and well-established link to C which is replicated in WG1. This is despite separate pots for legumes and other plants, meaning the OM already present in the soil is the likely source of any N-driven effect on C. N is driving differences in mid- and low-lability C but not in active labile C or total C.

N is a significant predictor of recalcitrant C. It is not a significant predictor of labile C, which is the fraction most C change is driven by. N is directly or indirectly related to a process which has some influence on the recalcitrance of C

3.6.3 Species

Chicory can adapt its rooting strategy to the C and nutrient levels in its growing medium.

Yarrow is the only species significantly driving differences in any fraction of C. Total LOI is influenced by yarrow but no other species, and not whether the soil is control (bare) or covered. Yarrow is linked to changes in labile C and total LOI, indicating a significant impact on soil C. However, a lack of research on the life history of the plant means the implications of this result for use in land management strategies are limited.

Simply covering the soil with a cover crop is not enough to stop C losses from a C-rich soil as the sole intervention. The functional groups tested in WG1 are not enough to prevent C losses from a C-rich soil. Control bare earth pots were not significantly different in C to pots containing plants, meaning that in such a high nutrient loaded soil, plant-related fluctuations were likely negligible because the plants caused very little change, likely because inputs and outputs were comparable

3.6.4 Depth

It is a key finding from the WG1 experiment that difference in labile SOC is mostly driven by changes in the top 10 cm soil. There is a relationship between lability and shallowness in WG1, but no evidence to establish a link between recalcitrance and depth.

No depths other than 10 cm showed any link to total or fractionated C. However, most C losses are driven by losses in the most labile fraction.

Mid-lability C also showed no links to any depth but 10 cm, illustrating a relationship with microbially-produced C, but indicating a low likelihood of ley treatments creating a lasting effect on deeper and/or more recalcitrant C.

No depths are related to C in its most recalcitrant form. No evidence has been produced over a two-year period in high-C soil for a relationship between soil depth and the presence of less bioactive C

3.6.5 Time

Only September 2020 (the third sample, after seven months' development) is a significant predictor of recalcitrant C, with no grading of sample month, indicating fluctuations in C which are not particularly seasonally or annually predictable from a limited dataset (five replicates, two years). Recalcitrant C is less expected to vary seasonally than labile.

Total LOI responds to the month in which the sample was collected, indicating a seasonal change or change over time driven mainly by labile C.

3.6.6 Roots

No relationship between root biomass and C was observed. No relationship between root biomass and depth was observed. With variations in rooting morphology, shorter or thinner roots than usual due to plentiful nutrient supply, and a short timescale, patterns in root biomass were hard to distinguish.

3.6.7 Management implications

Funding C leys of these species is not an effective use of finance directed solely towards improving soil C concentrations. However, there is some benefit to establishing a ley in terms of SOC, even if the difference is not significant over two years in an already C-rich soil. Using leys in combination with other managements including limited or no tillage for the duration of the ley and then shallow ploughing the cover crop back in as green manure could be helpful for increasing SOM on a short timescale such as that of a temporary agricultural ley, generally around 2-5 years. However, cover cropping alone is not enough to prevent all soil C loss. These managements can be used on C-rich soils to prevent further loss but there should be further investigation on low-C soils to establish whether the C content of the soil initially should have a bearing on the recommended managements in terms of ley establishment diversity, timescale, and accompanying management such as green manuring or mulching.

3.6.8 Limitations

The direct and indirect effects of measuring BD mean that some responses may have changed due to the act of measuring them. Disruptions caused by repeatedly sampling the same location might be resolved in a future experiment by drilling the same number of holes in the pots as the intended number of samples. Other options might include a raised bed with removable slats and dividers, rather than separate pots with one access point; however, a design with removable or hinged sections proved too difficult to create. Also, disruption will only be minimized, and not removed.

The C-rich soil of WG1 had some negative impacts on the applicability of the experiment to C-depleted soil. Plants grew vigorously without the usual limiting effect of low N and C, but this affected rooting systems (particularly observable in chicory), which does not provide a result representative of *in situ* effects on most C-depleted agricultural soils. It is on these C-depleted soils where research into plant-derived carbon benefits would be most applicable. This has been rectified by using a different soil for the second mesocosm (Chapter 4).

Patterns in normal plant-mediated C change were also more difficult to identify in a soil already rich in C. Plants did not have to synthesise as much of their own C from the atmosphere as they might in an average herbal ley, because of the plentiful supply in the soil of WG1. This means that plants were at times a net drain on soil C rather than a net contributor. This may not be the case in a C-depleted soil because plants may allocate more resources to synthesizing C from the atmosphere which may then be transferred to the soil by way of rhizodeposition. This was learned from for WG2 experimental design.

However, herbal leys and grasslands are not soil recovery methods solely adopted on C-poor soil, and therefore this experiment has the benefit of focusing on healthy soil, which is a key source of GHG emissions (H. X. Wang *et al.*, 2013; Chang *et al.*, 2021) including from peaty soils which are often still under farm management (Freibauer, 2003; Ostle *et al.*, 2009).

3.6.9 Suggested future research

Future research in a similar experiment should include measuring some aspect of plant growth without killing the plant, e.g. leaf surface area, height, or main stem diameter, as this was an aspect missing from the WG1 experiment. This data could have helped to support conclusions drawn from root data and, particularly in the case of chicory, contributed to better understanding growth patterns with respect to soil C.

The result of yarrow significantly predicting both labile and recalcitrant C was unexpected, given similar rooting strategy to timothy and similar reproduction with rhizomes and seeds to other plants in the experiment. There is very little research on the life history of yarrow and its exudates, seasonality, rooting morphology, and other physical and chemical parameters which may help explain this interesting result and mean it can be usefully interpreted for managing land for improved soil C.

There is a lack of data on soil C processes below 20-30 cm depths, which the design of the WG1 mesocosm aimed to incorporate. However, further research into deep soil horizons and the effect of surface managements on these is needed to design management strategies and funding opportunities which directly positively influence soil C and create incentives for managing the whole soil resource sustainably.

WG1 likely had an abundant nitrifying microbial community because of the predicting effect of nitrate on C; however, microbial communities were not analysed. This would be another change to make if a similar experiment were to be repeated, as we are missing key data on microbial abundance and type. Abundance was attempted with acid precipitation and using spectrophotometry which did not yield good results. Bioinformatics might be a more effective route for further similar experiments to explore as microbes are important drivers of key soil processes.

There is the potential to take this experimental design further in terms of investigating active land management strategies for C improvement. These include soil flipping where low-C soil is placed at the surface and the C-rich O horizon is buried away from increased air and water supplies, which encourage microbial activity and exposure to O_2 (Schiedung *et al.*, 2019). This would require much more soil and space to enact as a mesocosm and may be better addressed as a field experiment. Another approach which could be replicated in a similar mesocosm experiment would be ploughing in the cover crop as a green manure (Powlson *et al.*, 2012). These were not investigated in the WG1 experiment but may help return some C to the soil, rather than growing plants simply to cut them off and measure soil parameters without some aspect of returning plant matter. This is a valid replication of many agricultural systems where OM is removed from site including hay or sileage harvesting from meadows and pasture. However, focusing on management for soil C, adding the OM would increase the knowledge on available soil C conservation strategies, by incorporating managements such as cover cropping for green manure. There is support in the literature and in centuries of practice for some form of plant-derived OM return to soil to provide a longer-term positive effect (Rickman *et al.*, 2002; Premi *et al.*, 2013; Herrighty *et al.*, 2021).

Chapter 4: Grassland plant-mediated effects on concentration of soil carbon in a mixed species herbal ley (second Walled Garden experiment)

Abstract

Emissions from the top 15 cm soil are equivalent to the GHG reductions made from other areas in the UK. Soil C loss is an under-acknowledged contributor to C emissions, which adds to atmospheric C concentrations and depletes important soil nutrients.

Herbal leys incorporate more biodiversity than grassland leys. Biodiversity has been linked to increasing soil C and improved soil health. Temporary leys are often in rotation for short periods, 1-5 years, and subsidised by governmental funding for environmental land management schemes. Evidencing herbal ley function for soil health improvements could incentivise further scheme expansion and contribute to meeting soil health and GHG emissions reductions targets.

This mesocosm was designed to mimic the regenerative agricultural practice of a herbal ley incorporating diverse functional groups, assessing herb and grass species individually and in combination, to address the following hypotheses:

1. That low-carbon soil shows a greater improvement in SOC stocks than C-rich soil
2. That plants grown in low-carbon soil have deeper rooting systems than those in C-rich soil
3. That functional diversity correlates to an increase in SOC stock
4. That the presence of a legume increases C storage

The low-C soil of WG2 showed an overall C loss, although an increase in proportion of C stored in more recalcitrant fractions. Species drives labile C and recalcitrant C change, but not mid-lability or total LOI. Depth was not an influence on any C fraction, although a highly significant predictor of root biomass. Time progression was a very good predictor of labile C although weaker for mid-lability C and not for recalcitrant or total C change. Root biomass was a predictor of labile and mid-lability C, although not recalcitrant or total C, and was greater in WG2 than WG1. The presence of legumes did not improve C storage in the soil or the rooting depth of accompanying species. Functional diversity did, however, lead to the greatest root biomass and a slight increase in total C by LOI (%) in ribwort plantain + red clover + timothy, and ribwort plantain + timothy treatments, although the differences are not detectable in the model.

4.1 Introduction

Studies of SOC have mostly focused on the top 15 cm of soil, as this is where the majority of C losses are concentrated: up to 13 million tons of carbon were lost from this O-A horizon every year in the UK between 1978-2003 (Bellamy *et al.*, 2005; Schulze and Freibauer, 2005). This is almost equivalent to the reduction in emissions achieved each year during the same time period (Schulze and Freibauer, 2005). It is also equivalent to 8% the UK's emissions from other sources during this time, although not generally accounted for under GHG emissions data as soil emissions are not considered under the Kyoto Protocol (Schulze and Freibauer, 2005).

The UK happens to have some of the best-studied soil in the world, by region, geology, land-use and over a long period of time (Schulze and Freibauer, 2005). Despite this, there are still clear gaps in the literature where depth, cover species, time and legacy effect are concerned. These gaps in knowledge affect the policy decisions influencing the agricultural landscape, which incentivise changes to common agricultural practice which can influence the collective capacity for soil carbon sequestration (Bateman *et al.*, 2013). Currently, policies which do not take full account of the potential for regenerative agricultural practice may prevent optimising existing strategies for meeting national and international carbon targets (Bateman *et al.*, 2013; Soussana *et al.*, 2019). However, it is difficult to make arguments for time-specific interventions when only partial information is available (Paloma *et al.*, 2013). For example, there is research on the effects of sward diversity in temporary herbal leys (Jordon *et al.*, 2022), but there is a lack of information on the time over which these take place, or the legacy of the effect within a rotation cycle, or depth effects (Dalal *et al.*, 2003; Jarvis *et al.*, 2017; Reid, 2019).

Soil C losses are proportional to existing carbon content, which implies a decay of a homogenous pool and contradicts the theory that C-rich soil automatically has a higher fraction of stable carbon (Bellamy *et al.*, 2005; Schulze and Freibauer, 2005). However, correlation has been established between stand age of woodland and initial SOC content, which are predictors of deep soil SOC change after afforestation of agricultural soils (Shi *et al.*, 2013). The Shi et al. (2013) study also showed that the O horizon has an important role in carbon sequestration after afforestation of agricultural sites. However, this refers to afforestation rather than herbal leys; the same may not necessarily be true in herbal leys, and there is a lack of research in this area of carbon pools and the systems by which carbon is transformed to more or less reactive states.

It is important to examine soil under a range of carbon concentrations, as change in the top 15 cm soil can range from -66 g C m^{-2} y^{-1} uptake in very carbon-poor soils, to a loss to the atmosphere of +550 g C m^{-2} yr^{-1} from peat soil (Bellamy *et al.*, 2005; Schulze and Freibauer, 2005). The more depleted the soil is at the start of the process, the greater its initial C storage capacity, but this tends to be slow and plateaus over time to a carbon steady state (West *et al.*, 2004; Lal, 2005; Eze, Palmer and Chapman, 2018). The inclusion of a C-poor soil for WG2 following the establishment of WG1 on a C-rich soil was intended to address these potential differences in C change.

Herbal leys are a generally accepted method of sequestering more soil carbon than arable crops (Jordon *et al.*, 2022). Any plants are better than none when the land is to be left fallow: when plant growth was suppressed in an experiment at Rothamsted, UK, this produced a marked decline in soil C and aggregate structure (Hirsch *et al.*, 2017). Restoring grasslands to pre-industrial levels of biodiversity can sequester carbon, but this has only been established on much longer timescales than current leys are maintained for – up to a century (Poeplau et al., 2011; Yang et al., 2019). The Rothamsted experiment took 50 years before distinct differences were observable (Hirsch *et al.*, 2017). However, after 22 years, C storage rate (not total soil C) in high diversity fields is double the rate of storage in low diversity fields, associated with increased aboveground and belowground biomass production (Yang *et al.*, 2019). Mimicking natural succession processes such as a fire regime on former arable fields, Fornara & Tilman (2008) achieved a 500% increase in soil carbon over just 12 years when comparing diverse leys to monocultures. Diversity, especially when including N-fixing legumes, encourages the processes by which C and N are assimilated into the soil which can create a positive feedback effect (Zhao *et al.*, 2020). A reduction in a limiting factor, usually N, can allow plants to allot more resources to belowground biomass production (Fornara and Tilman, 2008), which is linked to the processes by which invertebrates and microbes bring carbon into the subsoil biosphere, with the breakdown of rhizodeposits away from the surface (Mellado-Vázquez *et al.*, 2016; Dhamala *et al.*, 2017).

The depth to which this sequestration is effective is obviously a key concern when putting these managements into practice, when soil can be metres deep but anything below 30 cm – the depth of a plough – is under-researched (Rasse *et al.*, 2006), and where anything deeper than 10 cm is 'deep carbon' in some studies (as in Shi et al., 2013). Investigations into soil carbon have suggested that conservation tillage, the use of altered tillage depth or frequency with the goal of improving soil health, may distribute residues into the plough depth at 30 cm rather than remaining concentrated in the top 5 cm, but that this is a distribution change rather than total carbon stock increase (Piccoli *et al.*, 2016).

Rhizodeposition, the excretion of exudates, gases, and dead tissue from plant roots, is a fundamental player in C cycling. It is a rapid process for incorporating C into microorganisms and SOM (Pausch and Kuzyakov, 2018). Around 50% the carbon assimilated by plants is transferred below ground, some into building and provisioning roots, but some also into rhizodeposition (Rees *et al.*, 2005). However, its speed of transition from plant exudates into the microbial biosphere means it is a difficult process to study and quantify, despite its importance (Pausch and Kuzyakov, 2018). The relative contributions of root death and exudation, and the processes by which products of these are assimilated into the soil, remain unclear (Rees *et al.*, 2005). It is likely that a more diverse plant species assemblage expands the variety of exudates and the depths and timescales over which they are exuded (De Deyn, Cornelissen and Bardgett, 2008). Increased species diversity in a herbal ley has a positive effect on the rate of microbial NH_4^+ consumption and inorganic N immobilisation (preventing some N flux to the atmosphere), which could be due to rhizodeposition stimulating microbial growth (Lama *et al.*, 2020). However, a mix of herbal species is not necessarily correlated with a growth of microbial community, diversity or abundance (Stefanowicz *et al.*, 2022). The potential for deeper carbon sequestration with deeper-rooting perennials including *Cichorium intybus* and *Plantago lanceolata* (chicory and ribwort plantain) has been investigated (Cooledge *et al.*, 2022), under the assumption that deeper rhizodeposits away from reaction with air and moisture may feed into soil SOC stocks (Dodd *et al.*, 2011).

Herbal leys have also been accepted as providing other benefits to soil health, such as aerating the soil to the point that sub-soiling should be unnecessary, and some additives such as lime might be made redundant after three to four years of consistent establishment (Brunetti, Morris and Keilty, 2006). Plants improve aggregate structure, and perennial untilled fields support different groups of microorganisms (Hirsch *et al.*, 2017). Deep rooting plants, which have been investigated for their potential for deeper rhizodeposition influencing deep soil carbon (Cooledge *et al.*, 2022), also create pores for air and water deeper underground. This may improve conditions which also increase C assimilation at depth, such as altering pH and water content to a more hospitable environment for a microbial community to assimilate C (Dodd *et al.*, 2011).

The presence of deep-rooting herbs can reduce the nitrogen fertiliser requirement (Cooledge *et al.*, 2022). Grasses rely on the N synthesised by legumes, rather than N available in the soil, when grown in a diverse ley (Dhamala *et al.*, 2017), indicating a possible way to fill the N gap while reducing artificial N inputs. The use of fertilisers, alongside fossil fuels, has hugely increased reactive nitrogen in the soil, which may help plants sequester more C in the soil and in their tissues by overcoming the usually limiting factor of N supply (Crowther *et al.*, 2019). However, there are significant drawbacks to supplying N for agriculture, and an excess of N in the environment. Nitrogen has very low use efficiency, with over 50% the total reactive N load emitted to the atmosphere (Gu *et al.*, 2012). The production and shipping of fertiliser is a C-intensive process and contributes to indirect agricultural emissions; as much as 1.2% the total global GHG emissions from the consumption of energy is the result of fertiliser production (Kongshaug, 1998; Wood and Cowie, 2004). Hypoxic 'dead zones' in waterbodies develop as a result of N pollution, including one of the largest in the Gulf of Mexico which is 20,000 km^2 and largely the result of leaked N inputs from farms channelled down the Mississippi (Robertson and Vitousek, 2009). Nitrogen has a potential annual cost across the EU27 of €75-485 billion, around 60% of which is related to air emissions, with these costs calculated from impacts to the environment and human health (Van Grinsven *et al.*, 2013). In China, the cost to health from N emissions to the atmosphere reached US$19-62 billion in 2008 (Gu *et al.*, 2012). As a result, agricultural developments to reduce N inputs, which can be conducted on a wide scale, across many farming systems and climates, have the potential to create many positive changes in the food production system.

The presence of herbs can also help promote the recovery of soil macroinvertebrate populations in degraded agricultural soils (Cooledge *et al.*, 2022). The removal of the plant community correlates with a drop in macroinvertebrate abundance, with bacteria five times more abundant in grassland than arable fields and ten times more than bare fallow (Wu *et al.*, 2012; Hirsch *et al.*, 2017). There are strong links between plant diversity, and soil biodiversity and soil-derived ecosystem function and services (Delgado-Baquerizo *et al.*, 2020). Soil invertebrates decompose and redistribute SOM, and contribute to supporting soil structure (Rana and Khan, 2010). They also consume soil microbes, affecting the microbial structure and thereby the balance of C and nutrients assimilated in the microbial community (Wardle, 2006). Modern agriculture has an established negative effect on soil structure, gas exchange and natural recycling processes, all of which negatively affect the soil macroinvertebrate community (Rana and Khan, 2010; Hirsch *et al.*, 2017). There is a positive relationship between biodiversity and ecosystem function across biomes, including in soil, with diversity supporting a more resilient function (Fitter *et al.*, 2005; Delgado-Baquerizo *et al.*, 2020), so it is important to investigate measures which could help support greater diversity in the soil biota. A greater diversity of genus and species are generally found in lower-input fields than higher-input fields (Rana and Khan, 2010). SOM influences earthworm abundance, and tree cover influences the abundance of organisms which function as litter fragmenters (Huerta and van der Wal, 2012). Another way of improving the invertebrates on a site, including in soil, is through regenerative parasite management. As mentioned above with regards to livestock health, plants which produce metabolites such as tannins and sesquiterpene lactone are effective at reducing internal parasite burdens (Mueller-Harvey, 2006) and therefore anthelmintic use can be reduced (Hayward, 2021). Anthelmintics in livestock farming decrease invertebrate abundance in dung beetles, which are valuable cyclers of OM into the soil, which also reduces the need for fertiliser (Hayward, 2021). Understanding how managements affect diversity is important when the assemblage supports a variety of ecosystem services and contributes towards sustainable soil health; adapting land management towards allowing a greater variety of soil invertebrates to flourish will support a positive feedback loop of greater C storage and nutrient cycling (Griffiths *et al.*, 2021).

Soil microorganisms are crucial drivers of soil ecosystem services (Strecker *et al.*, 2016), but rely on plant material entering the soil biosphere from autotrophs – that is, plants (Eisenhauer *et al.*, 2010). When plants are suppressed in fallow areas, it correlates with a marked decline in microbial abundance (Hirsch *et al.*, 2017). Plant species richness increases soil microbial community abundance over time, and plant functional group diversity influences the spatial stability of soil microbes (Strecker *et al.*, 2016). However, increased plant diversity requires at least four years before significant impacts on microbial diversity can be observed (Eisenhauer *et al.*, 2010). The recovery of the microbial community under herbal leys converted from monoculture to a more natural state can take over a decade (Strecker *et al.*, 2016). There is also a tipping point of plant diversity (which requires two to four years to reach, depending on diversity) where the microbial community will transition from an unstable and inefficient community of zymogeneous (producing enzymes or inactive precursors to enzymes requiring another process to access the active site) microorganisms which react to pulses of inputs, to a more stable and efficient autochthonous (representative of the natural and original) community (Eisenhauer *et al.*, 2010). Other ways plants can influence the microbial community include affecting soil permeability to water and inputs; creating air and water pores by the action of their roots; exuding N; and by affecting the soil pH (Fromin *et al.*, 2012). Higher soil pH is related to lower SOC storage (Chen *et al.*, 2018). Excess reactive N in the soil can reduce microbial activity which sequesters C in the soil, possibly outweighing the benefit of increased plant growth as a C sink (Crowther *et al.*, 2019). However, a small addition of N can lead to increased SOC as N can be a limiting factor for microbial growth (Eisenhauer *et al.*, 2010).

Leys are also useful for farm productivity, as there is evidence that perennial forbs in addition to grass species in a ley improve livestock productivity (Jordon, Winter and Petrokofsky, 2022). Every measure which improves farm productivity per ha is contributing to a reduction in the environmental impact per unit of food produced (Herrero *et al.*, 2016; Jordon, Winter and Petrokofsky, 2022). Legumes improve the tonnage of forage production per ha with their N-fixing properties, with a sward including legumes producing 1.63 t ha^{-1} more than a ley without legumes, and a legume-only sward produced 2.2 t ha^{-1} more than a ley without legumes (Jordon *et al.*, 2022). The heavy impact of legumes suggests it may not be solely species diversity which drives benefits, but functional diversity, or representation of functional traits; for example, including C4 pathway species with legumes brings a greater increase in SOC stocks than C3 plants with legumes (Fornara and Tilman, 2008). This is because C4 species use the N produced by legumes more efficiently, which increases their energy contribution to belowground biomass and thereby soil C and N (Fornara and Tilman, 2008).

Herbal leys are also one component of regenerative agriculture practices which are promoted on the basis that they improve forage production (Jordon *et al.*, 2022). When considering the overall impact of the inclusion of herbs and perennials on soil carbon storage, it is important to include their contribution to livestock welfare, as this is also a priority for farmers, and in law and trade. There is evidence that the varied diet of a herbal ley improves livestock performance by widening the range of micronutrients the animals have access to (Brunetti, Morris and Keilty, 2006), and through the natural properties of some plants, such as worm suppression evidenced in reduced faecal egg counts (Kidane *et al.*, 2010; Peña-Espinoza *et al.*, 2016; Grace *et al.*, 2019). With legume diversity increasing leaf nitrogen concentration, sheep daily weight gain increased by 3.5 g d^{-1} compared to a diet without legumes (Jordon *et al.*, 2022). Cattle have been shown to increase their milk yield on a more varied diet compare to a *Lolium perenne* (perennial ryegrass) diet (Loza *et al.*, 2021). The generally deeper-rooted herbage also enables greater biomass production through access to water in dry periods, compared to ryegrass, which improves provisioning for livestock year-round (Cooledge *et al.*, 2022). Diverse pastures can also provide higher crude protein than monoculture (Jerrentrup *et al.*, 2020). Adding 20% ribwort plantain seed in a perennial ryegrass-clover mix can result in an increase in dry matter yield as high as 9.5% (Cong, Søegaard and Eriksen, 2016). Increased finishing weights have been demonstrated in cattle grazing on birdsfoot trefoil, and in cattle and sheep grazing chicory (Kidane *et al.*, 2010; Macadam and Villalba, 2015; Peña-Espinoza *et al.*, 2016).

A more varied diet including herbs has provided evidence of a reduction in livestock enteric emissions (Loza *et al.*, 2021; Cooledge *et al.*, 2022). Agriculture contributes 47% global CH_4 emissions, a GHG which has 25 times the GWP of CO_2 (Piñeiro-Vázquez *et al.*, 2015), and therefore any measure which can produce even a small drop in the emissions of an individual animal will have a substantial cumulative effect if practiced on a global scale. CH4 is produced by fermentation in the gut, which is influenced by the gut flora (Piñeiro-Vázquez *et al.*, 2015). The presence of tannins and metabolites specific to certain plant species may help reduce enteric CH_4 emissions (Loza *et al.*, 2021), as these compounds can affect rumen microbes. Condensed tannins can reduce protozoa populations by up to 79% and thereby decrease methanogen production by up to 33% (Piñeiro-Vázquez *et al.*, 2015). Tannins may suppress rumen CH_4 production by up to 58%, as well as increasing weight gain in the animal because of an increased flow of less-digestible protein to the small intestine, or by reducing the proportion of energy lost to producing CH_4, which can reach 12% the cow's energy intake (Piñeiro-Vázquez *et al.*, 2015). When a production cycle is considered as one holistic system, the impacts on enteric emissions and productivity are also important considerations because an increase in milk yield may be cancelled out by an increase in enteric emissions, for example (Loza *et al.*, 2021).

Alongside animal health as an animal rights and welfare matter, there is also the consideration of preference: while perennial ryegrass is the most common cattle forage plant, in a selection of 35 sown plots of different forage species, ryegrass was the least grazed by a herd of Jersey cattle, which preferred sheep's parsley, ribwort plantain and chicory as the top three, in that order (Turner, 1974; Brunetti, Morris and Keilty, 2006). Two of these species are examined in the WG2 experiment below.

Acknowledging these potential benefits while conscious of the gaps in the literature casting doubt on the longevity of the effects of a soil under rotational management and over the timescales of achievable carbon increase, I have designed a herbal ley microcosm. The second Walled Garden experiment (WG2) takes representatives of three functional groups: legumes, grass, and a mid-to-deep-rooting herb, to examine the single and combined effects of these plants on the carbon and basic parameters of a depleted agricultural soil. Limitations on experiment size and sampling process time meant functional groups was also a way to incorporate diversity without resorting to species diversity, which would create an unmanageable number of experiments.

The experiment aims to address these hypotheses:

5. That low-carbon soil shows a greater improvement in SOC stocks than C-rich soil
6. That plants grown in low-carbon soil have deeper rooting systems than those in C-rich soil
7. That functional diversity correlates to an increase in SOC stock
8. That the presence of a legume increases C storage

4.2 Methods

4.2.1 WG2 establishment

I filled 60 small plant pots with SOM-rich topsoil and placed a pinch of seeds in three depressions in each pot. This was to create a surplus of plants, as the pots were under variable shade and wind exposure conditions along the outside wall of a greenhouse, and sheltered by the original WG1 experiment. The species planted were timothy grass (*Phleum pratense*), red clover (*Trifolium pratense*) and ribwort plantain (*Plantago lanceolata*); these three plants were chosen to cover the grass, herb and legume categories, while ribwort plantain also maintains a thick and rhizomatous root (although not as distinct a taproot as dandelion or chicory, the mixed thick and fibrous rooting system is known to reach depths of up to 1 m (Pol et al., 2021). 20 pots of each species were established. After three weeks and bad weather, only around half the seeds had germinated, so a second round of planting was carried out on 26th May 2021. Throughout, when necessary, the plants were watered. These plants germinated successfully.

In contrast to WG1, this experiment sourced low-C soil from a turf farm, under the assumptions that a lower C baseline would show fluctuations in C more clearly; that lower C soil is more representative of the soils that herbal leys might be sown on to help improve their health in situ; and that plants would grow in a more representative way of a real farm scenario, i.e. exploratory rooting systems which create more rhizodeposits, rather than the unnaturally vigorous growth and reduced rooting systems seen in the C-rich WG1 soil. The reduced rooting systems of WG1 may have reduced rhizodeposits, due to a lesser requirement for a comprehensive rooting system with resources more readily available.

The plants were transplanted from the small pots of SOM-rich topsoil into the 1m-tall pots of C-depleted soil for long-term establishment on 12th July 2021. Before allocation, the plants were uprooted, keeping the roots as intact as possible by first loosening the soil. As much soil as possible was then removed from the root matrix by brushing out delicately with fingertips, which leaves the inoculum of current root associates intact. The plants were thus allocated:

- The five largest plants of each cohort were weighed, their roots measured from the base of the plant to the tip of the longest root, and then transplanted into their own pots. The weight of the large plants was recorded, with the exception of timothy grass, the dense root matrix of which prevented a significant amount of soil removal, thereby likely affecting the weight measurement so much as to be of little use.
- Plants were then grouped into clusters. Pots which would require two species had 'medium' sized plants or clusters (a higher frequency of small plants necessitated 'clusters' of 2-3 to balance out approximate size and weight) and pots which would require three species had 'small' plants or clusters assigned. Each plant/cluster would be visually assessed to ensure approximately equal distribution between the 2- and 3-species pots.
- An attempt to weigh them to increase the accuracy was attempted but the basic scales used in the field were not precise or accurate enough to determine the weight of such small plants, and therefore visual comparison was used. However, the root length could still be measured, from the base of the plant/cluster to the tip of the longest root present.

After measurements were taken, the plants were transplanted into the tall pots via a randomised system. I listed each combination 1-40, in sections of the five replicates. I then used a random number generator to generate numbers 1-40 and listed these, corresponding to the pots in a line left to right and continuing along the other side (Table 4.1). The approximate sizes and weights of the plants were used to combine them to approximately equally distributed 'bunches' in the long pots (Table 4.2).

Table 4.1. Randomly assigning positions to species combinations

Randomly generated number	Assigned species combination	Pot number
34	Timothy + red clover + ribwort plantain	1
5	Timothy	2
32	Timothy + red clover + ribwort plantain	3
10	Red clover	4
7	Red clover	5
14	Timothy + red clover	6
23	Timothy + ribwort plantain	7
20	Ribwort plantain	8
33	Timothy + red clover + ribwort plantain	9
19	Ribwort plantain	10
40	Control	11
24	Timothy + ribwort plantain	12
27	Red clover + ribwort plantain	13
12	Timothy + red clover	14
25	Timothy + ribwort plantain	15
37	Control	16
9	Red clover	17
2	Timothy	18
22	Timothy + ribwort plantain	19
36	Control	20
15	Timothy + red clover	21
38	Control	22
1	Timothy	23
18	Ribwort plantain	24
3	Timothy	25
30	Red clover + ribwort plantain	26
8	Red clover	27
16	Ribwort plantain	28
29	Red clover + ribwort plantain	29
17	Ribwort plantain	30
31	Timothy + red clover + ribwort plantain	31
28	Red clover + ribwort plantain	32
39	Control	33
4	Timothy	34
11	Timothy + red clover	35
21	Timothy + ribwort plantain	36
26	Red clover + ribwort plantain	37
35	Timothy + red clover + ribwort plantain	38
13	Timothy + red clover	39
6	Red clover	40

Table 4.2 Combining species by weight into multiple-species pots

Pot number	Species 1	Species 1: weight (if applicable) (g)	Species 1: root length (cm)	Species 2	Species 2: root length (cm)	Species 3	Species 3: root length (cm)
1	Timothy	-	23	Red clover	16	Ribwort plantain	16
2	Timothy	-	26	-	-	-	-
3	Timothy	-	15	Red clover	12	Ribwort plantain	14
4	Red clover	30	18	-	-	-	-
5	Red clover	40	16	-	-	-	-
6	Timothy	-	34	Red clover	24	-	-
7	Timothy	-	30	Ribwort plantain	15	-	-
8	Ribwort plantain	90	25	-	-	-	-
9	Timothy	-	5	Red clover	24	Ribwort plantain	15
10	Ribwort plantain	50	23	-	-	-	-
11	Control	-	-	-	-	-	-
12	Timothy	-	29	Ribwort plantain	19	-	-
13	Red clover	-	22	Ribwort plantain	20	-	-
14	Timothy	-	32	Red clover	26	-	-
15	Timothy	-	20	Ribwort plantain	17	-	-
16	Control	-	-	-	-	-	-
17	Red clover	20g	15	-	-	-	-
18	Timothy	-	12	-	-	-	-
19	Timothy	-	22	Ribwort plantain	19	-	-
20	Control	-	-	-	-	-	-
21	Timothy	-	24	Red clover	19	-	-
22	Control	-	-	-	-	-	-
23	Timothy	-	20	-	-	-	-
24	Ribwort plantain	60	36	-	-	-	-
25	Timothy	-	12	-	-	-	-
26	Red clover	-	27	Ribwort plantain	20	-	-

27	Red clover	50	25	-	-	-	-
28	Ribwort plantain	40	21	-	-	-	-
29	Red clover	-	24	Ribwort plantain	18	-	-
30	Ribwort plantain	30	21	-	-	-	-
31	Timothy	-	5	Red clover	15	Ribwort plantain	19
32	Red clover	-	20	Ribwort plantain	-	-	-
33	Control	-	-	-	-	-	-
34	Timothy	-	23	-	-	-	-
35	Timothy	-	32	Red clover	15	-	-
36	Timothy	-	21	Ribwort plantain	23	-	-
37	Red clover	-	26	Ribwort plantain	24	-	-
38	Timothy	-	7	Red clover	12	Ribwort plantain	13
39	Timothy	-	17	Red clover	23	-	-
40	Red clover	40	22	-	-	-	-

4.2.2 Sampling and timing – learning from WG1

Continuing to learn from the WG1 approach to the initial experiment, I was aware of the seasonal fluctuation in soil carbon with the growth periods of the plants. Biannual instead of quarterly sampling would allow the seasonality of carbon changes to still be observed, but manage the sampling flow and processing time of both WG1 and WG2 simultaneously. WG2 was sampled in February 2021, August 2021, and February 2022, providing 18 months' data covering seasonal change, coinciding with the length of a temporary ley towards the shorter end of a ley lifespan (leys in agriculture are generally established for 1-5 years).

4.2.3 Field sampling methods

Additionally, the experiment was destroyed at the end of its life, in August 2022, to give root data in relation to depth increments. Three out of the five replicates were sawn into 10 cm cores. The plastic piping was marked at the actual soil surface level, as pots had sinkage ranging from 4-7 cm. Then the top 2 cm of soil were also marked off as the range where stem and leaf tissue and other detritus would also be present. From there down, the pipe was marked at 10 cm intervals. The final interval was 80+ cm, rather than continuing as 80-90 and 90-100 cm, as few pots had more than five cm soil below the 90 cm mark.

4.2.4 Laboratory processing

For this experiment, the laboratory processing included pyrolysis for C in three fractions of lability; all the basic parameters such as bulk density, water content and pH; and ion chromatography for nutrient content. The roots of three out of five replicates were also analysed. The detailed methods for these can be found in Chapter 2.

The initial stages of processing were also completed for C:N analysis (rolling 0.05 g dry soil into aluminium foil, preparing standards, spreadsheets for accompanying weights and labels) and for VOAs (filtering, centrifuging, processing with 0.1 mol KCl solution and orthophosphoric acid). However, these are not yet processed for current time of analysis and writing. C:N samples were delayed for the same reasons as WG1 January 2022: Covid-19 affecting access and training, staff sickness, and a broken machine. VOAs were delayed in August-November due to problems with gas supplies, machine and software issues, and then a backlog of samples in a queue. Unfortunately, this means initial analysis has been conducted without these elements, but they have been paid for so will be incorporated into analyses at a later date.

4.2.5 Statistical analysis

Generalised linear models (GLMs) were conducted on a dataset where all outliers and obviously incorrect data (e.g. weight loss on ignition >100%) were removed. GLM was chosen as it can cope with both categorical and continuous variables, and linear and non-linear data.

Categorical variables, which are month, depth and species, were dummy coded and then written as factors to force R to recognise them as categorical.

Root data, like WG1, was a smaller dataset. Multiple linear regression in R set total C % as the response variable against the predictors of root weight (g), depth (cm) and species. Time was taken out of the equation as roots can only be compared when they have a known measurement, i.e. at death, because the replicates were destroyed for the data.

4.3 Results

Four GLMs were run against the LOI at a temperature interval, indicating loss of organic matter in labile, medium and recalcitrant fractions. The LOI interval was the response variable and other parameters the predictors: month (May or July 2021, or February 2022); plant species in isolation or in combination; water %; nutrients in the form of nitrate, nitrite or phosphate; pH and BD.

4.3.1 GLM_250

GLM_250 refers to a GLM where the response variable is the most labile fraction burned off at 20-250^{o}C in the muffle furnace. The results are as follows:

- The deviance residuals are more spread out than WG1, with 1Q at -7.03, median at 0.76, and 3Q at 4.75.
- The third and final sample month, that of February 2022, had a significant influence on how much labile carbon was in the soil, $p = 0.011$
- Every plant option, except the baseline and triple-species combination, showed up with significance:
    - Red clover & ribwort plantain (RCRP): p = 0.006
    - Timothy & ribwort plantain (TRP): p = 0.024
    - Timothy & red clover (TRC): p = 0.018
    - Ribwort plantain (RP): p = 0.007
    - Timothy (T): p = 0.019
    - Red clover (RC): p = 0.016
    - Control (C): p = 0.017

- There were no significant influences from nutrient content, water, pH, or in the first two months of the experiment

4.3.2 GLM_325

GLM_325 refers to the model where the response variable is the carbon lost on ignition between 250-325ºC. The results are as follows:

- The deviance residuals are very close but slightly uneven: 1Q = -1.198, median = -0.430, 3Q = 0.9296
- The second month, i.e. the first sample month after the baseline (samples taken in July 2021 after establishment in May 2021) showed a significant influence to *p* = 0.018
- In February 2022 the *p* value remained significant at 0.021
- However, for this medium lability carbon, only ribwort plantain had a significant influence, *p* = 0.013
- Water was also a significant influence on the medium lability carbon concentration, *p* = 0.029
- Phosphate also showed a significant relationship, *p* = 0.041
- pH, nitrite, nitrate, BD, and none of the other species showed any significance

4.3.3 GLM_550

GLM_550 refers to the response variable of LOI at 325-550ºC. The results are:

- the deviance residuals are the most spread out and furthest median from 0 so far: 1Q = -11.74, median = -1.29, 3Q = 6.18
- Only the timothy and ribwort plantain combination of plants has any significant impact: p = 0.036
- No depths are significant, in line with GLM_250 and GLM_325
- No other variables are significant

4.3.4 GLM_Total_LOI

GLM_Total_LOI refers to the model run with total LOI as the response variable as a proxy for total C. It is less accurate than C:N analyser-measured C as non-C compounds are also burned off in the furnace, but has the benefit of indicating fractions, as discussed above. The results of the total LOI model are:

- Deviance residuals of 1Q = -21.17, median = -3.34, 3Q = 7.52 (a larger range and more uneven than previously)
- Nothing shows as significant when all temperature intervals are included together

4.3.5 Root_GLMs

Root_GLM refers to the models analysing the predicting effect of species, root weight and depth intervals on total C. Total LOI values were matched to root data of the same replicate and depth interval. Root_GLM_1 analysed the predicting effect of these against total LOI; Root_GLM_2 analysed them against labile C; Root_GLM_3 analysed them against mid-lability C; and Root_GLM_4 analysed them against recalcitrant C. A further set of GLMs were run to examine the prediction ability of species, depth and carbon (total or by fraction) on root biomass (g). These were Root_GLM_5, 6, 7 and 8.

Root_GLM_1 results (total C):

- Deviance residuals of 1Q = -0.936, median = -00424, 3Q = 0.0496. Median close to 0 but some unevenness either side

- Timothy + ribwort plantain, timothy + red clover, timothy, and red clover are good predictors of total LOI. *P* values stand at 0.036, 0.010, 0.006, and <0.001 respectively.
- Depth and root biomass (g) variables did not show as significant

Root_GLM_2 results (labile C)
- Deviance residuals of 1Q = -0.931, median = 0.037, 3Q = 1.147. Some unevenness, median close to 0
- CP (*p* = 0.006), TC (*p* = 0.002), T (*p* = <0.001), and clover (*p* = 0.030) showed as significant predictors of labile C
- Root biomass (g) was a significant predictor of labile C, *p* = 0.024

Root_GLM_3 results (mid-lability C)
- Deviance residuals: 1Q = -1.134, median = 0.020, 3Q = 3.903
- CP (*p* = 0.006), TC (*p* = 0.003), T (*p* = <0.001), clover (*p* = 0.030) showed as significant predictors of mid-lability C
- Root biomass (g) was a significant predictor of mid-lability C, *p* = 0.027

Root _GLM_4 results (recalcitrant C)
- Deviance residuals: 1Q = -0.827, median = -0.160, 3Q = 7.323. Uneven, but median close to 0
- TP is a significant predictor, *p* = 0.004
- Root biomass (g) is not a significant predictor of recalcitrant C

Root_GLM_5 results (predicting root biomass from labile C, species and depth)
- Deviance residuals: 1Q = -1.060, median = -0.068, 3Q = 0.737
- 30 cm, 60 cm and 90 cm depth intervals were significant predictors of root biomass (g), with each producing a *p* value of <0.001
- Species not significant
- Labile C was also a significant predictor of root biomass, *p* = 0.024

Root_GLM_6 results (predicting root biomass from mid-lability C, species and depth)
- Deviance residuals: 1Q = -1.354, median = 0.018, 3Q = 0.929
- Depths significant (same data as Root_GLM_5, *p* values all <0.001 although different values because of different intercepts and variables interacting)
- Species not significant (same data as Root_GLM_5)
- Mid-lability C also a significant predictor of root biomass, p = 0.027

Root_GLM_7 results (predicting root biomass from recalcitrant C, species and depth)
- Deviance residuals: 1Q = -0.921, median = -0.084, 3Q = 0.877
- Depths significant, species not (same data as Root_GLM_5 and 6, depth *p* values all <0.001)
- Species not a significant predictor
- Recalcitrant C also not a significant predictor

Root_GLM_8 results (predicting root biomass from total LOI, species and depth)
- Deviance residuals: 1Q = -1.009, median = -0.091, 3Q = 1.043
- Depths all significant predictors, all *p* values <0.001
- No species are significant
- Total LOI is not a significant predictor

4.4 Discussion

4.4.1 Soil differences and sampling limitations

There was a textural difference between the soil of WG1 and the soil of WG2. The soil of WG2 was finer with a higher clay content and lower C content, influenced by the reduced load of OM. This made it easier to filter the soil of WG2 for roots than the soil of WG2 as they could be filtered using water, where the sediment sank and roots floated, and the soil was fine enough to sieve easily and retrieve the remainder the root biomass from each core. As discussed in section 3.4.1, differences in soil texture are correlational not causational for OM and C with particle scale influencing grain size and thereby soil texture. Particle size is a factor in the SOC storage potential of the soil, as increased clay content increases storage capacity, which is a positive indicator for the clayier soils of WG2. Mineralogy influencing C storage potential by way of influencing aggregate stability, seal formation and run-off (Lado and Ben-Hur, 2004; Kögel-Knabner *et al.*, 2008; Bhattacharya *et al.*, 2016) and the 'young' particulate matter loosely connected with soil minerals which is readily bioactive (Kaiser *et al.*, 2002). Respiration from SOM decomposition by the microbial community also influences C storage potential (Xu & Shang, 2016). It may be the case that, in this C-poor clayey soil, clay content influences C holding potential over mineralogy, with microbial respiration and SOM decomposition also playing a part. Run-off and soil loss would be less related to mineralogy in a pot with a raised lip and no base drainage, as in WG1 and 2, but the mineralogy may still influence aggregate stability, which was higher in WG2 than WG1 with less sinkage.

As discussed also in section 3.4.1, BD may not have been particularly accurate or representative of the whole pot due to sampling methods through pre-drilled holes causing resampling interference. Creating a physical space may influence rooting depth and pattern, microbial abundance or diversity, water transport, O_2 contact, and thereby indirectly C and N storage. I acknowledge that by measuring the outcome, I have potentially changed the outcome: BD itself may be inaccurate due to compaction or crumbling; roots may take a different path; and C and N are secondarily influenced by the introduction of space, air and potentially bacteria or other organisms.

Plant growth was not directly measured, although photos were taken. A comparison is shown below in Fig. 4.1 of WG2 over time. It was noted that the plants of WG2 grew much less vigorously than those of WG1, possibly due to lower C and nutrient concentrations in the soil. Designing WG2 with a lower soil C concentration aimed to address the difficulties in WG1 with observing patterns in soil already high in C, and with plant growth and root changes due to different limiting factors than would be expected *in situ*. In a low-C soil plants must assimilate more C from the air than they had to in the soil of WG1 with plentiful supplies easily accessible by even reduced rooting systems. Herbal leys on C-poor soil are likely to have a better pay-off in terms of increasing total SOC compared to a rich soil, as SOC losses to primary production and other processes such as erosion also tend to be proportional to existing C concentration (Schulze and Freibauer, 2005). This experiment is designed to be more representative of the capabilities of a functionally diverse plant community to sequester carbon in the soil by way of rhizodeposition, over an appropriate timescale for leys in agroindustry.

Figure 4.1 The WG2 experiment three months post-establishment (PE) in August 2021 from the east, (top); three months PE from the north (mid left); nine months PE in February 2022 from the north, when final soil samples were taken (mid right); and one year PE from the east when the experiment was destroyed for the roots in August 2022 (bottom).

4.4.2 GLM_250

Basic soil parameters

Interestingly, water % was not a predictor of labile C, despite water content being an important mediating factor in several processes which affect soil C, such as microbial activity (Churchman and Tate, 1986; Fitter *et al.*, 2005), plant growth (Puget and Drinkwater, 2001; Pausch and Kuzyakov, 2018), and nutrient transport (Jiao, Whalen and Hendershot, 2006; Ruiz-Colmenero *et al.*, 2013). This could be because there was little fluctuation in water levels over time, with a wet summer and a dry winter, which did not match with seasonal effects of plant death or senescence when root tissues die off and add to subsoil OM. Water may never have been a limiting factor on plant or microbial activity forming labile C in WG2 and therefore is not showing as a good predictor. However, water did have a significant predicting effect on mid-lability C, which is further discussed below. Without differentiating time, species or depth effect, the relationship between water (%) and labile C by LOI (%) is illustrated in Fig. 4.2 below, showing a linear effect with a weak positive correlation.

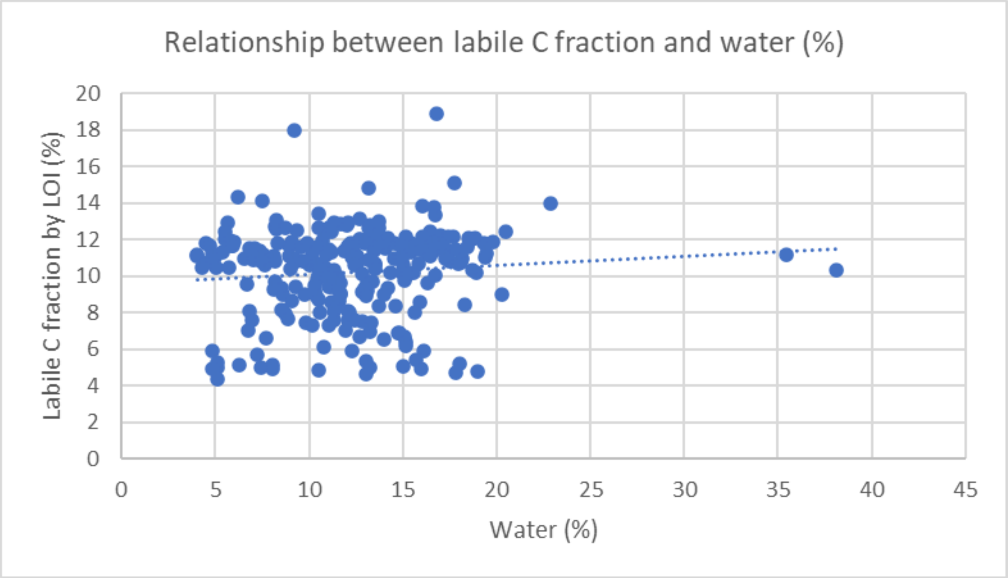


Figure 4.2 Linear relationship between water (%) and LOI (%) as a C proxy

Labile C was not significantly responsive to pH in WG2. Labile C concentration did drop when planted, compared to baseline C pre-ley planting (Fig. 4.4, 4.5, 4.6) although this did not show up as significant in the GLM. This may be related to nutrients having no influence over labile C either, in such a short period of time (one year). It could simply be that there has not been enough time to see an impact of pH or nutrients on labile C, as the breakdown processes which produce N- and P-containing compounds from OM inputs influence pH, usually increasing the acidity of the soil with humic and fulvic acids (Bayat *et al.*, 2021). These processes take place on a timescale greater than plant growth seasons, depending on other influencing factors such as OM inputs and water demand, and accumulate humus compounds over time. Fig. 4.3 illustrates the weak negative correlation between labile C by LOI (%) and increasing pH.

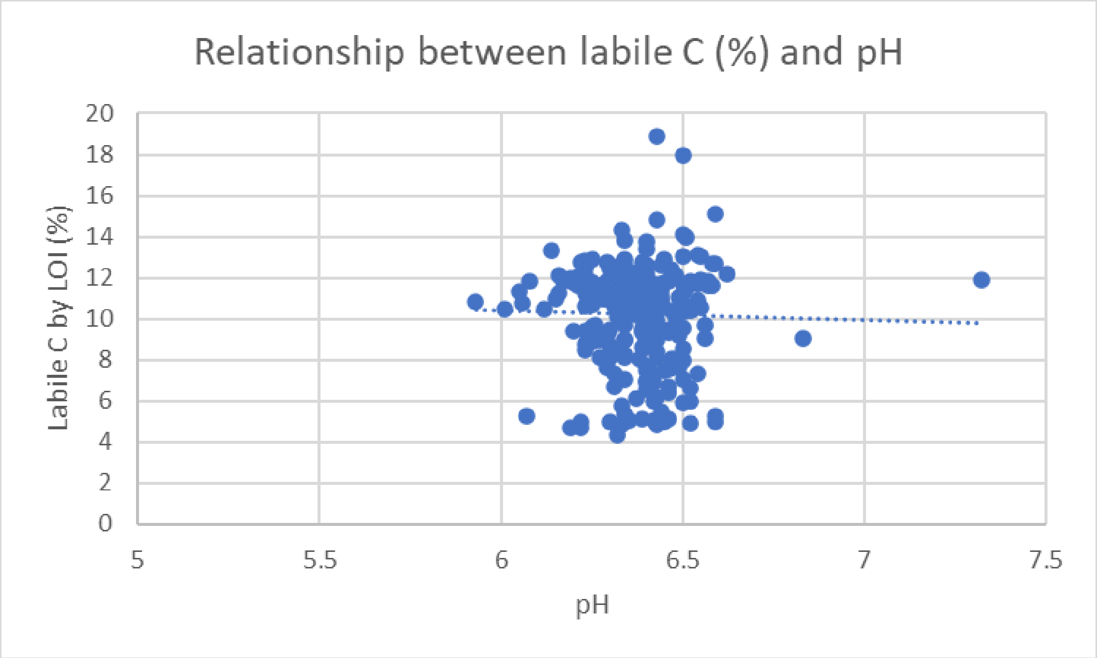


Figure 4.3 Labile C by LOI (%) and pH

There was no significant predicting effect from nitrite (see Fig. 4.4), nitrate (see Fig. 4.5), phosphate (see Fig. 4.6) or bulk density on labile C in the GLM. This is despite an established relationship in the literature between BD and C, and N and C. These influencing variables are mediated by other factors including water (%) (Wilson and Warren, 2015), the effect of plant roots on aggregation and pore creation (Lal and Kimble, 2000), and depth (Jurgensen *et al.*, 2017). These factors influence each other and thereby soil C (total or fractions) directly and indirectly. They are all also influencing factors on the microbial community, and important driver of labile C change (Zhang *et al.*, 2022). That none of the factors showed as significant predictors of labile C means their effects are either interacting to ameliorate each other rather than amplify each other, or that the timeframe of the ley representation is not long enough to observe a significant driving effect of nutrient concentrations or BD on labile C. An effect on labile SOC may accumulate over time but WG2 indicates that a normal ley lifespan is not enough to observe a positive influence of nutrient concentrations or establish a relationship between C and BD.

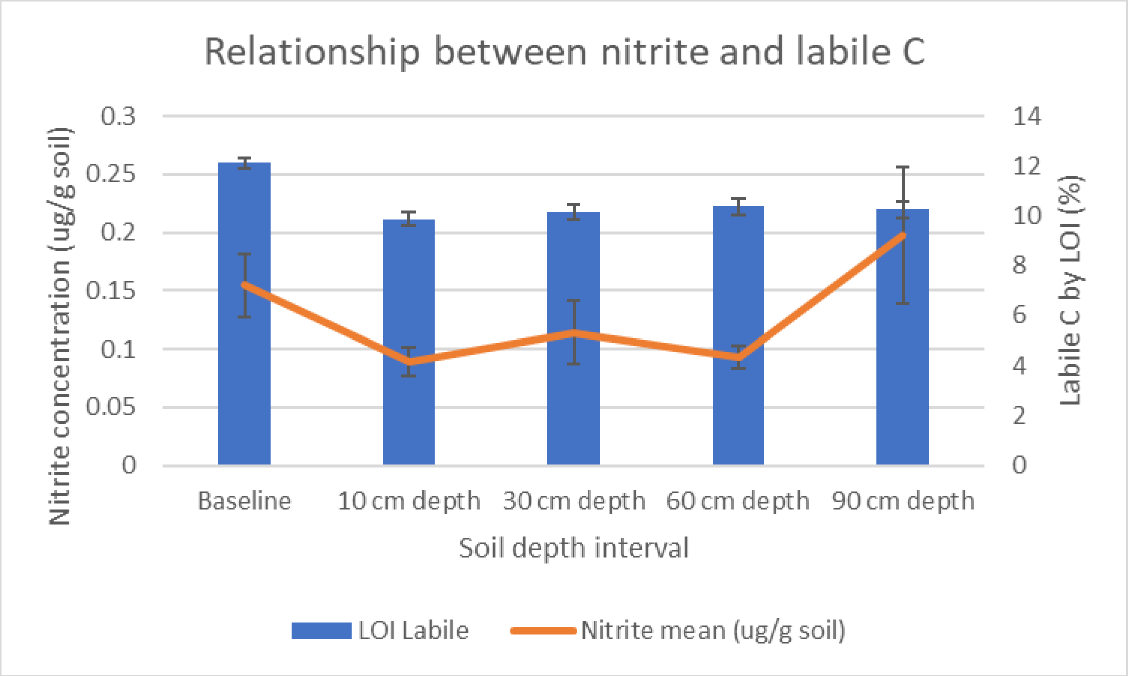


Figure 4.4 Representation of the lack of correlation between labile C (%) and nitrite concentration (µg/g soil)

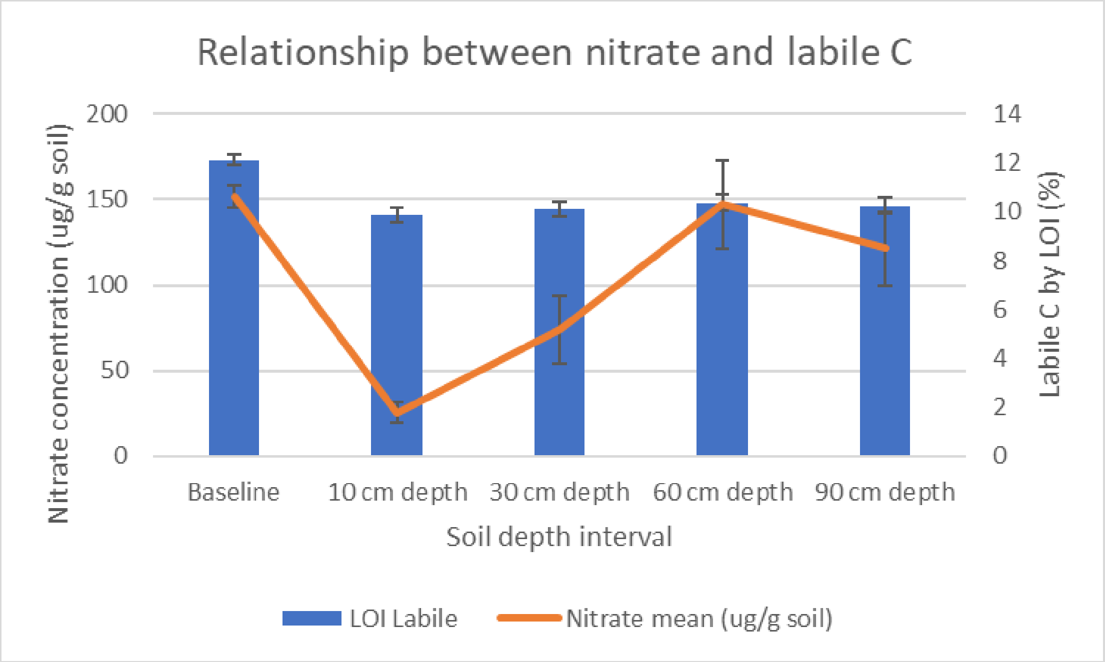


Figure 4.5 Representation of the lack of correlation between labile C (%) and nitrate concentration (µg/g soil)

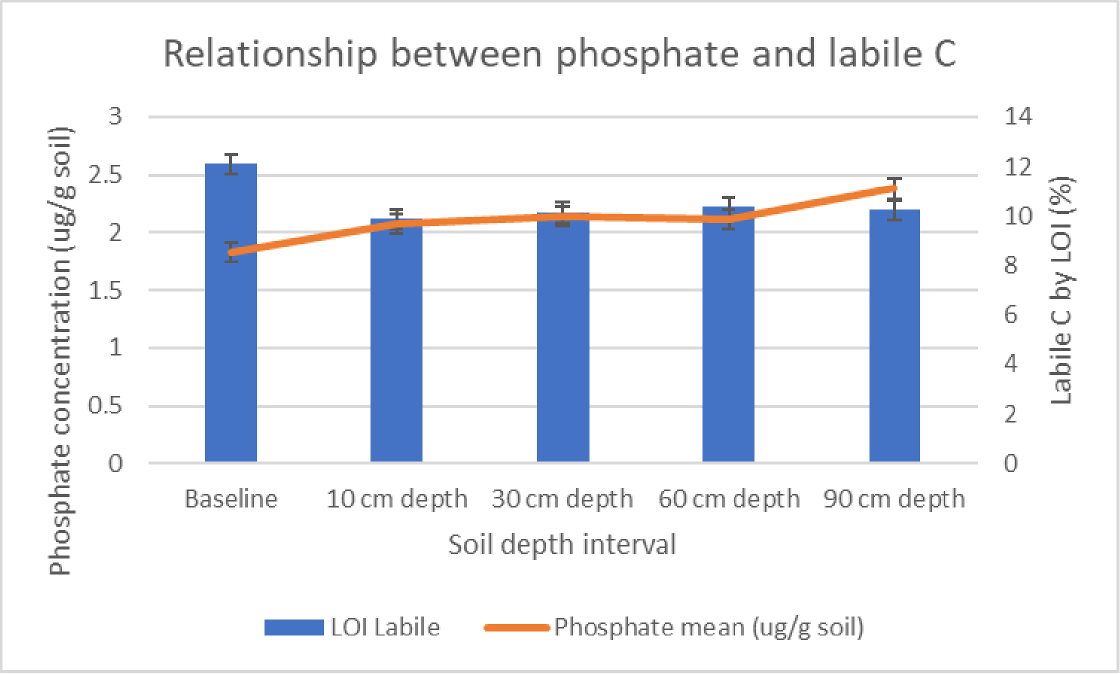


Figure 4.6 Representation of the lack of correlation between labile C (%) and phosphate concentration (µg/g soil)

Time progression

After nine months' establishment, by February 2022, time showed as a significant predictor of labile C. This is expected, as C changes over time with seasonal changes in plant growth and rhizodeposition. Labile C declined between the baseline and the first sample month (a three-month interval) and again between first and second sample month (a six-month interval). This is illustrated below in Fig. 4.7.

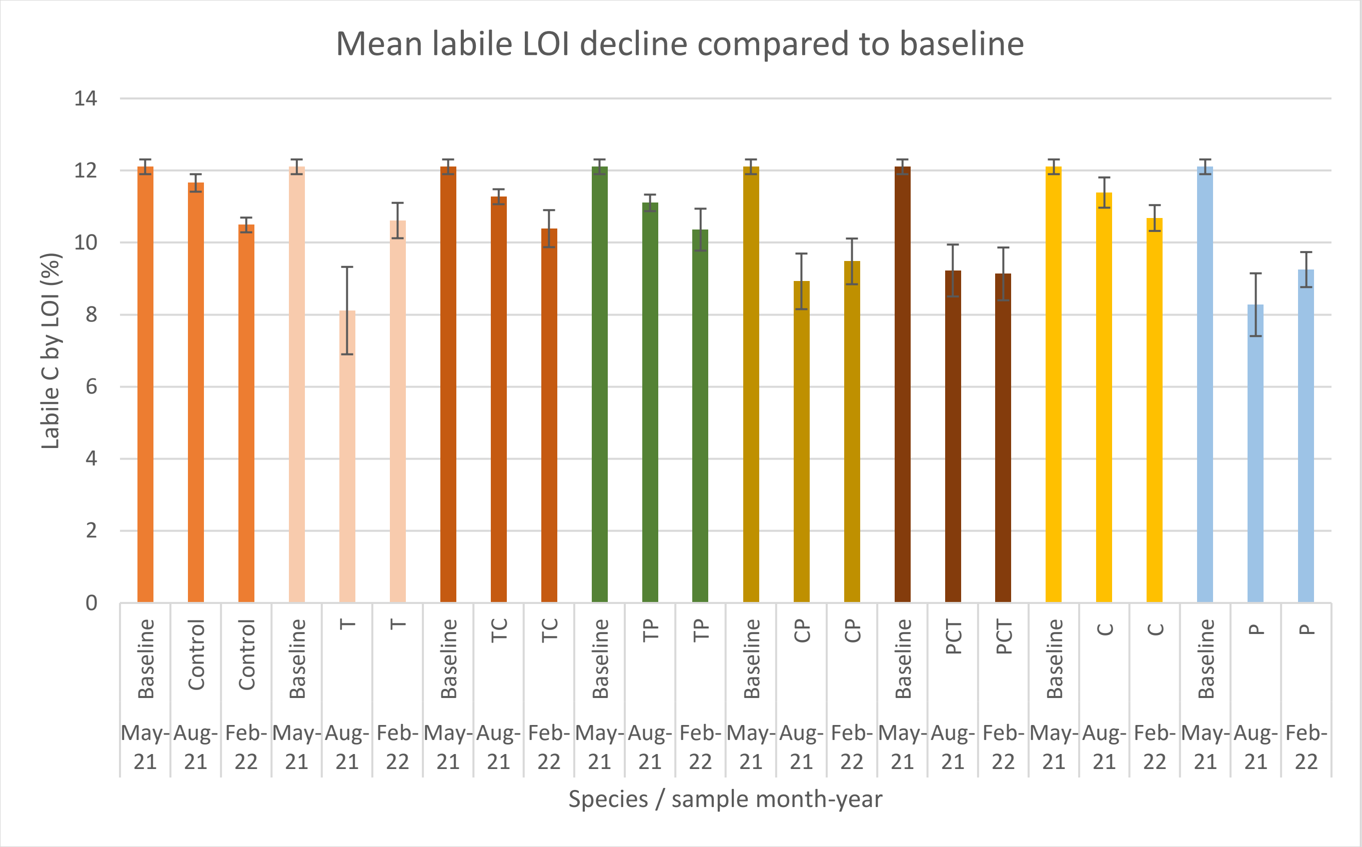


Figure 4.7 The drop in labile C between baseline and sample months across all species combinations and controls in WG2. T = timothy, TC = timothy + clover, TP = timothy + ribwort plantain, CP = clover + ribwort plantain, PCT = ribwort plantain + clover + timothy, C = clover, P = ribwort plantain

Fig. 4.7 illustrates the overall drop in C compared to the baseline, seen across every species including controls. Baseline mean was 12.10% labile C, dropping to a mean across all pots of 9.99% in August 2021, with a slight increase (although still a decrease compared to pre-planting) to a mean across all pots of 10.04% in February 2022.

Control saw the least reduction in labile C after three months, but still saw a drop from 12.10% to 10.49% over the course of one year. While some of this is due to the fact bare earth will see a greater loss of C from erosion from wind exposure and a lack of root stabilising effect (Evans, 1997; Barré *et al.*, 2010), we can surmise that not all the labile C decline seen in other pots is due to take-up by plants for biomass production with an estimate of up to 1.60% labile C being lost to the environment in other ways such as CO_2 flux, erosion, or leaching.

Control retained the most labile C after three months, which is expected because no plants are assimilating it into biomass. The next most efficient plants in terms of retaining labile soil C are clover, the soil under which decreased from 12.10 to 11.39% LOI; timothy + clover, which decreased to 11.27%; timothy + plantain, which decreased to 11.10% LOI; the three-species combination, which decreased to 9.23% LOI; clover + plantain, which decreased to 8.92% LOI; plantain, at 8.28%; and timothy grass at 8.11%. Although these species differences did not show as significant in the GLM, given time, the trends might become more defined. That timothy and ribwort plantain sit at the bottom for labile C retention after three months, but with the addition of a pairing plant are in the top three cover plants for C retention, implies there is a positive effect of biodiversity on labile soil C retention. This could be by way of interacting root systems which have different morphologies, complementing each other by accessing different pools of C or accessing exudates from the other species. However, the positive effect of biodiversity on soil C is not supported when three species are grown together in one pot, which could be because of higher demands in one C pool or depth with two or more root systems competing. The addition of N derived from the legume may also positively affect C storage in the soil as clover and timothy + clover are the top plant communities for labile C storage. There is an established link in the literature between C and N as N is usually a limiting factor on plant growth and biomass production (Loubet *et al.*, 2011; Crowther *et al.*, 2019). Grasses (in WG2, timothy) are particularly reliant on N synthesized by legumes rather than available in the soil (Dhamala *et al.*, 2017). Increasing N supply in low-C soil by organic means, including legumes, can help reduce C emissions and produce a net storage effect over several years by reducing soil respiration rates

However, time is only a predictor of labile C and not other C fractions, meaning that most change over time is being driven by changes in labile C. It also indicates that one year is not enough time to observe a significant difference in mid-lability or recalcitrant C. It is an important finding that there is a decrease over nine months in labile soil C, as this may inform managements for improving soil health from the perspective of crop growth, for example, targeted OM inputs. Plants access labile, bioactive C more easily than recalcitrant C in the soil but only part of their total biomass is produced from soil C as plants synthesise C compounds from CO2 in the atmosphere. Over a greater period than 12 months, if some of the trends observed in Fig. 4.7 towards increasing labile C after an initial drop continue, including plants such as timothy, red clover and ribwort plantain in a herbal ley could reduce the need for artificial inputs when the ley is rotated back into arable crop production. This has many benefits in terms of reducing emissions from the production and transport of fertiliser, reducing reliance on trade monopolies of the elements in fertiliser, and reducing the effect that applying artificial fertiliser can have on the long-term fertility and structure of soil. Therefore, the cumulative effect of emissions reductions is further-reaching than a small addition to total SOC stock. This positive effect of growing a diverse ley on soil C may also be complimented with the addition of further management strategies including green manuring the herbal ley into the soil rather than harvesting it for hay, to ensure OM is returned to the soil.

Similarly, earlier sample times were not predictors of labile C because of the time that C-forming and C-influencing processes need to develop. This is, however, an important finding. This means that while a one-year herbal ley may decrease C concentration in the soil as evidenced in WG2, the processes that inform soil structure, robustness to change, and build compounds related to C and soil health, can be influenced over one year. One year under a minimally managed ley is not enough to rehabilitate soil to increase labile C from the evidence in WG2. A ley alongside other managements might have a discernible positive impact (green manuring, soil flipping, green mulch, OM inputs), which requires further research. Other questions remain over the time a ley needs to be established; the frequency a ley must be planted in rotation to maintain soil C and nutrient levels; and the most effective companion managements.

Depth

Depth was not a predictor of labile carbon content. This does not add to the evidence provided by WG1, although WG1 was established for a longer period of time. There was no link between lability and shallowness as there was in WG1. Neither experiment showed a link between recalcitrance and depth. The GLM did not show any significant influence of depth on labile C but as illustrated in Fig. 4.8, patterns did emerge.

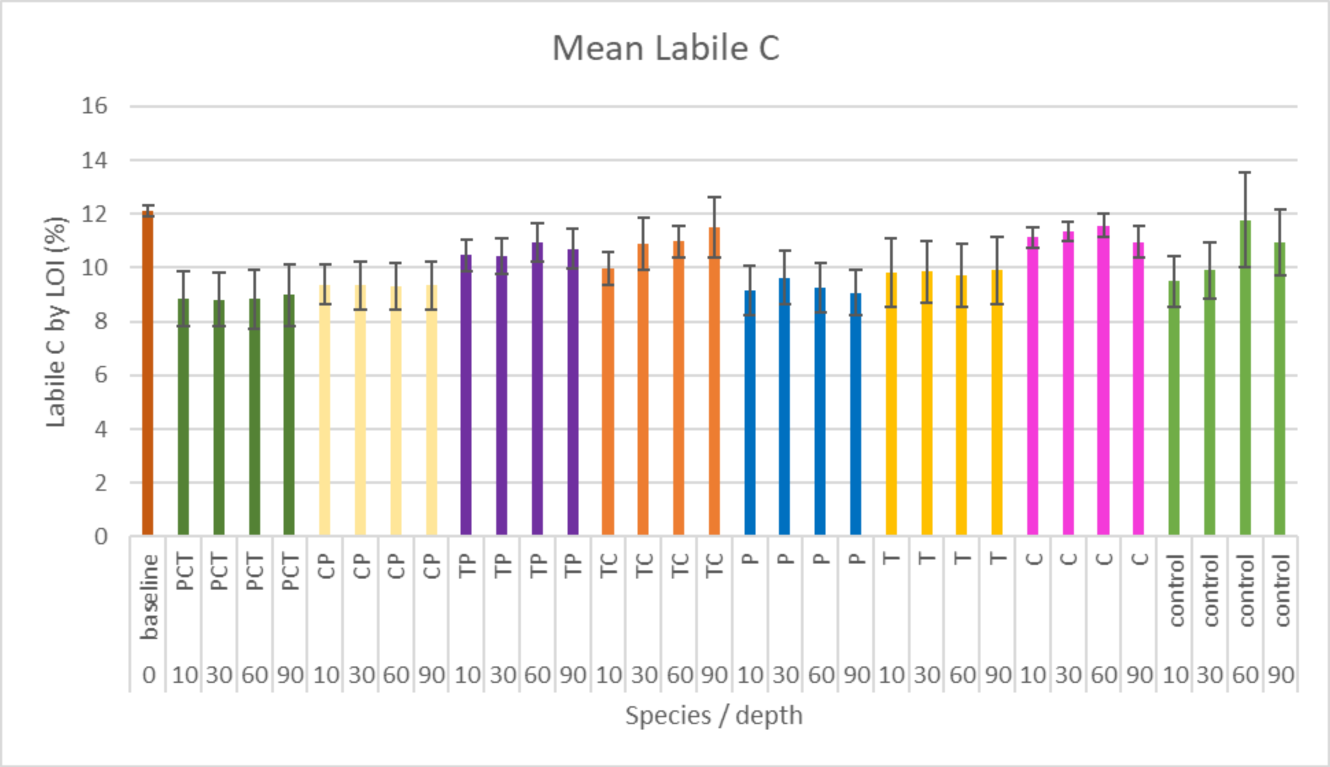


Figure 4.8 Labile C by LOI (%) through the soil depth profile according to plant species/ combination

The overlapping standard errors indicate the non-significant differences between depths, which the GLM established. However, broad patterns are visible. Timothy + plantain (TP) and timothy + clover (TC) store slightly more of the labile carbon in their soil at deeper depths, and timothy (T) and ribwort plantain (P) both had more C in the soils where they were one of a pair of species than in the soil where they were single species. Control pots had the widest fluctuations in labile C by depth, interestingly, suggesting plant species have a mediating effect over depth by accessing deeper pools with deeper roots, with labile C tapped into to resource biomass production over all depths. This compares to control pots where the only mediating effects are erosion, weather, and water transport.

Interestingly, TP and TC combinations demonstrate the opposite to the hypothesised effect of more labile C in shallow soil, where they have stored larger proportions of labile C at lower depths. While this may show a depth partition effect on labile C under the influence of plants, all combinations still demonstrate a net loss of labile C at every depth compared to baseline soil. Over a period of nine months, all the plants and plant combinations tested absorb more C from the soil than they return via the products of photosynthesis, senescence and rhizodeposition. This is important information with regards to the lifespan of temporary leys, where a priority of labile carbon increase would necessitate a longer lifespan than a year according to evidence from WG2 (and longer than two years according to evidence from WG1).

<u>Species</u>

Every plant and every plant combination, bar the triple-species pots, were significant predictors of labile C in the GLM. Table 4.3 below shows the change with each species over time. There were a few individual replicates which showed a gain in labile C (i.e. more than 12.10% LOI at 250^{o}C) but most saw a drop and every species/combination showed a mean drop in labile C over the nine months of the WG2 experiment. However, some of this initial decrease in labile C was recovered in some species. CP, T, and P all showed some recovery of labile soil C by February 2022 compared to August 2021, showing that some species/combinations are more likely to return labile C to the soil on shorter timescales than others (such as PCT, TP, TC, or C). It is expected that plants will initially take up soil C for biomass production, especially in spring/summer, but WG2 provides evidence that a ley in very short rotation (<1 year) is not adequate time to return C, even with species diversity. The evidence from WG2 shows that the most species- and functionally-diverse group, PCT, had the greatest net loss of C over nine months despite the presence of a legume expected to catalyse increased C deposition by other plants (Fornara and Tilman, 2008). Literature on increasing species diversity to improve C content in soil are usually on much longer-term studies (Fornara and Tilman, 2008; Cong, Søegaard and Eriksen, 2016; Cooledge *et al.*, 2022; Jordon *et al.*, 2022), so further research needs to address the temporary leys, established for five years or less, which make up over 20% UK grasslands (DEFRA, 2021b).

Table 4.3 Change in labile soil C by LOI (%) according to plant species/combination with standard error (SE)

Species/ combination	**Baseline (% LOI)**	**Mean labile LOI (%) August 2021**	**Mean LOI (%) February 2022**	**Mean net change over nine months (%)**
PCT	12.10±0.21	9.23±0.72	9.13±0.73	-2.97±0.19
CP	12.10±0.21	8.92±0.77	9.48±0.63	-2.63±0.35
TP	12.10±0.21	11.10±0.23	10.36±0.58	-1.74±0.52
TC	12.10±0.21	11.27±0.21	10.39±0.51	-1.72±0.70
T	12.10±0.21	8.11±1.21	10.61±0.49	-1.49±0.50
P	12.10±0.21	8.28±0.87	9.25±0.49	-2.85±0.38
C	12.10±0.21	11.39±0.42	10.68±0.36	-1.42±0.33
Control	12.10±0.21	11.66±0.24	10.49±0.20	-1.61±0.56

That the most important variables (time and species) show up in only the labile fraction GLM indicates that any carbon difference over time is still driven by fluctuations in the most labile fraction, and not because plants are particularly storing any carbon at depth or in recalcitrant fractions. Tying this into WG1, as WG2 never showed any depth significance, this is also therefore likely to be in the top 10 cm of soil, although the patterns are not strong enough in WG2 to indicate a depth influence.

4.4.3 GLM_325

Water

Water was a significant predictor of mid-lability C, which is a more expected result than the no-significance produced in the 250_GLM. As water content is an important mediating factor in several processes which affect soil C, it is expected that there is a link between C and water, but unexpected that this should occur only in mid-lability. This could be because any possible fluctuation (or lack of) in water content aligned with the processes forming mid-lability C, and the more recalcitrant the C, the longer and more stable the processes that form it (Rovira and Vallejo, 2002; Lorenz *et al.*, 2007).

Water may sometimes have been a limiting factor on plant or microbial activity forming mid-lability C in WG2, and therefore shows as a predictor variable. A representation of the weak negative relationship between water (%) and mid-lability C (%) is below in Fig. 4.9, with a grouping of two sets of values at higher and lower LOI.

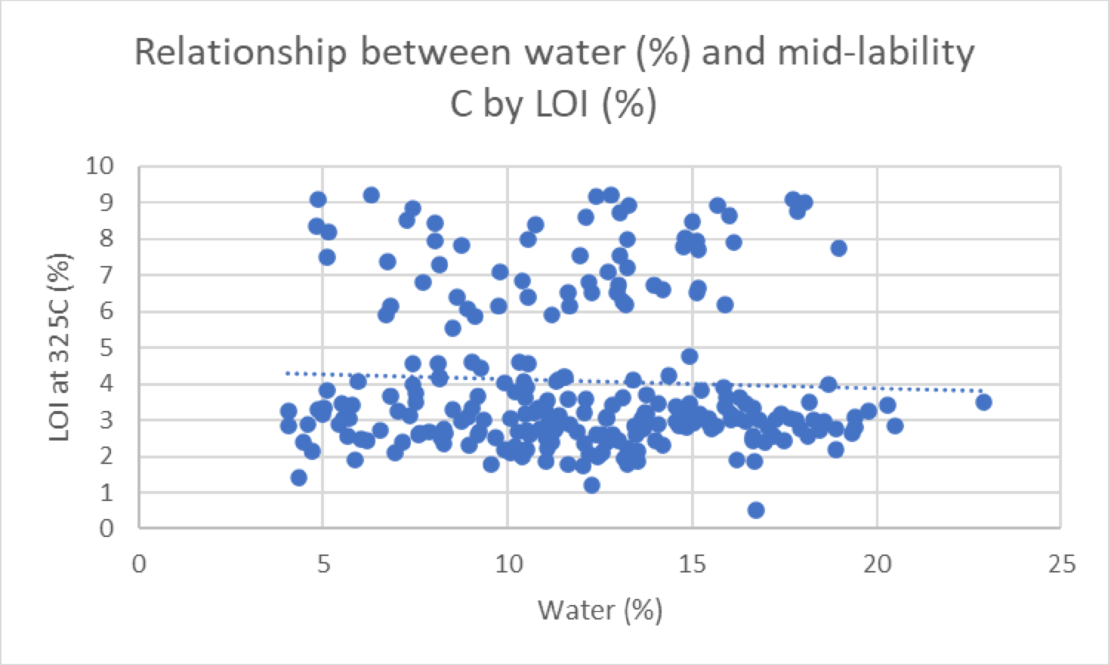


Figure 4.9 Correlation between water (%) and LOI (%)

<u>Nutrients</u>

Phosphate has a significant relationship to mid-lability C in the GLM. This may be because similar microbial communities are synthesising bioactive phosphate accessible to plants and mid-lability (still bioactive) C. The weak positive correlation between mid-lability C and water is shown below in Fig. 4.10. There are several potential explanations for this accurate GLM prediction of mid-lability C from phosphate, which are not necessarily mutually exclusive. The same microbial groups or processes may be producing one or both from the humus in the soil, which acts as a reservoir of both C and P (Stevenson and Cole, 1999). The ratio of C to P, usually 140:1.3 (Stevenson and Cole, 1999), either changed over time or species at a similar rate, or remained around the same, due to mirrored processes despite different plant species. Microbes play an important part in the humification of SOM from which both bioactive P and C compounds are derived; the microbes which produce them are separate taxa but rely on the same inputs, which were in good supply in WG2 (Hedley and Stewart, 1982; Stevenson and Cole, 1999). Phosphate is derived from soil parent material and mineralised, at the same time as C from soil parent material undergoes similar changes (Chai *et al.*, 2015). It could also be the case that phosphate producers have a symbiotic relationship with the carbon producers, as bacteria are major sources of C in soil (Ren *et al.*, 2018) and symbioses have been found between lichens (mycobionts and bacteria) and bacteria which solubilise phosphate (Sigurbjörnsdóttir, Andrésson and Vilhelmsson, 2015). Phosphate limitation is also used frequently in laboratory processes, although not well established in the field or in soil science, to limit cell growth which also reduces production of C-containing compounds (Schuhmacher *et al.*, 2014). There are multiple processes where phosphate and carbon cross paths but these are not particularly well explored in the literature and the predicting effect in the GLM could be the result of any of these processes or any number of interactions between them.

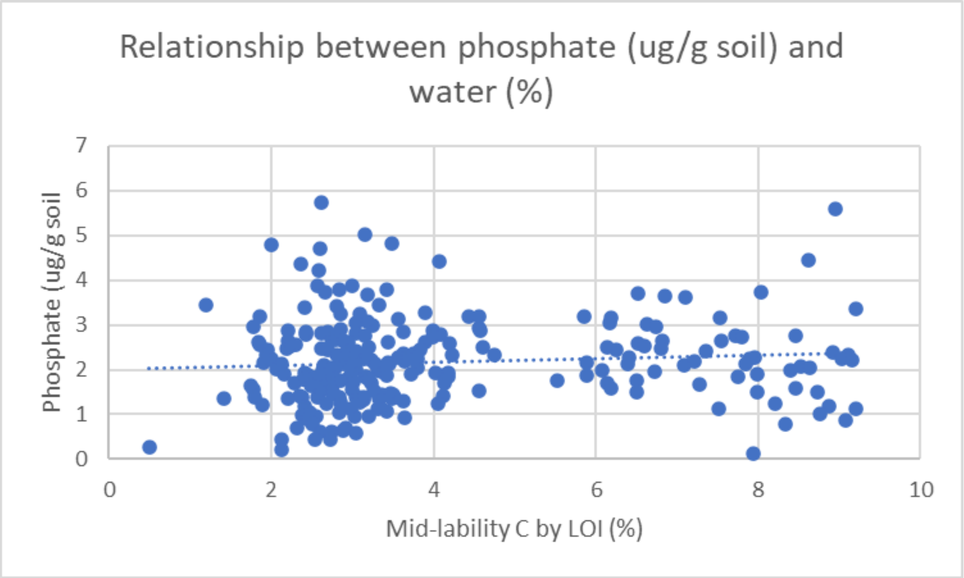


Figure 4.10. Relationship between phosphate (μg g soil^{-1}) and water (%)

Nitrate (see Fig. 4.11) and nitrite (Fig. 4.12) did not show any significant relationship to mid-lability C in the GLM, despite the fact that the production of bioactive N compounds from OM has the byproduct of increasing fulvic and humic acids, affecting C by means of pH change (Nelson and Bremner, 1969). Nitrate also acts as an oxidising agent, while nitrites are byproducts of ammonia degrading to nitrate. The lack of prediction strength in the GLM could simply be related to a smaller fraction of C being mid-lability compared to labile or recalcitrant, and therefore more difficult to identify patterns between it and other variables. Alternatively, it could be that these N compounds are transitive between labile and recalcitrant and don't persist as well in soil where C is in a state of medium lability.

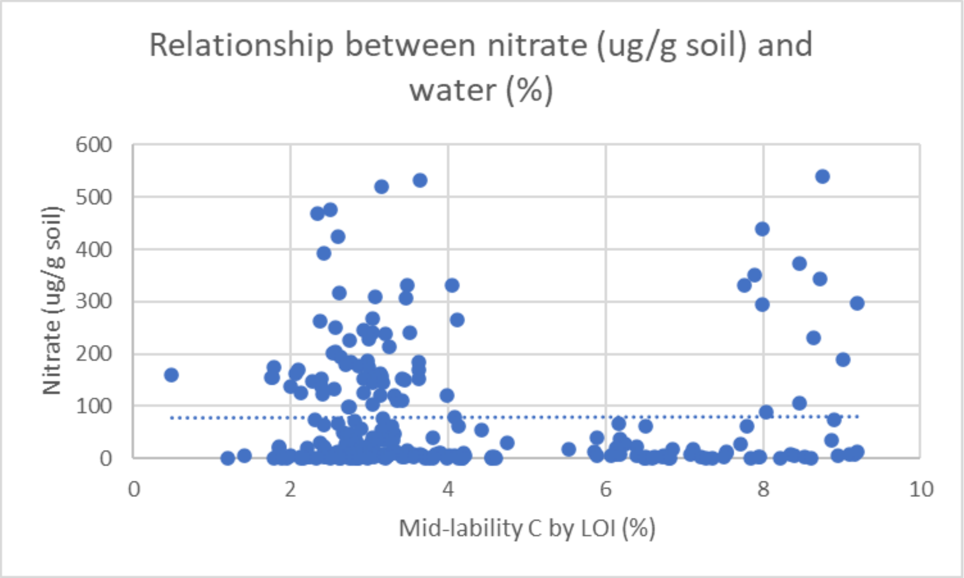


Figure 4.11 No correlation between nitrate and water

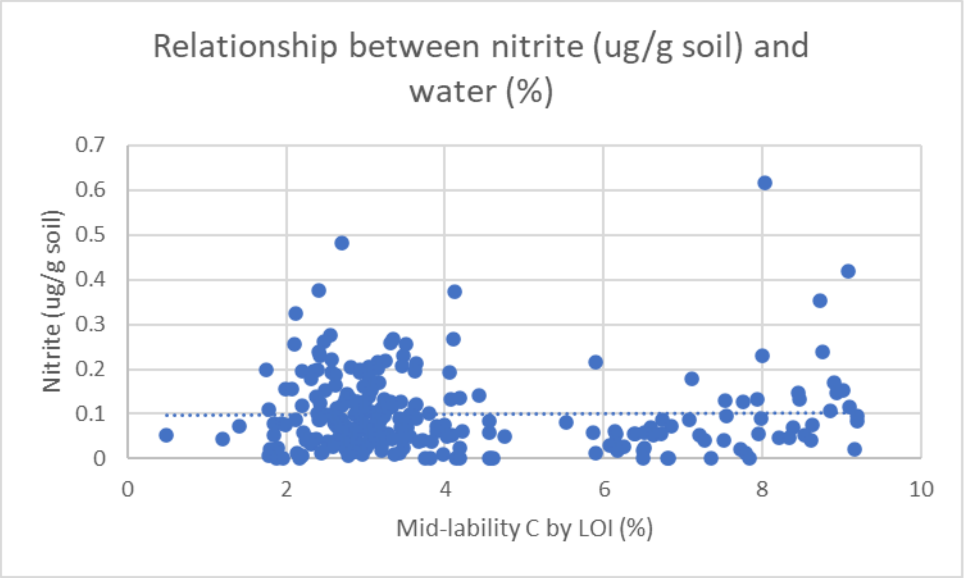


Figure 4.12 No correlation between nitrite and water

BD

BD showed no significant prediction of mid-lability in this GLM. This is despite an established relationship in the literature between BD and total C (Chambers, Beilman and Yu, 2010; Wilson and Warren, 2015; Jurgensen *et al.*, 2017), mediated by the rooting effects of the plant community, water content, and microbial processes (Lal and Kimble, 2000; Wilson and Warren, 2015). Land use including managements such as tillage, the use of other heavy machinery, grazing and cover cropping are also indicators of BD (López-Fando, Dorado and Pardo, 2007; Blanco-Canqui *et al.*, 2009; Liebig *et al.*, 2014; Wilson and Warren, 2015), which the literature focuses on as key field processes but which are not applicable to the WG2 experiment. Because this is a small temperature interval, it could be that less distinction in patterns around mid-lability C can be established in GLMs, and time is also a key indicator in soil processes and nine months may not be long enough to give clarity on some soil parameters. It is also important to note that under other management conditions including any form of tillage, relationship between C (in any fraction) and BD are likely to vary from the results presented from WG2.

pH

Likewise, pH showed no predicting ability for mid-lability C in this GLM. This may be related to the lack of relationship between nitrates and nitrites, because these compounds form through the decay of plant and animal tissues and ammonia through humification; this creates acids which are partially mineralised (Bayat *et al.*, 2021). It may be, again, that there is simply not enough time in a <1 year ley to observe differences which come from the breakdown of tissues, as the only plant inputs would be one season of rhizodeposition and leaf litter. However, this is important as leys may be harvested, leaving very little organic matter to be cycled back into the soil, similar to WG2. This means there is also scope for further research into how additional managements such as beating or ploughing a herbal ley into green manure could complement the carbon effect on the soil of growing it.

Time progression

Samples from August 2021, three months after WG2 was established, and from February 2022 after nine months, show time as a significant predictor of mid-lability C in the GLM. The progression of mid-lability soil C by LOI (%) according to species is illustrated in Fig. 4.13, where SE demonstrates significant differences. As Fig. 4.13 shows, every species and species combination tested in WG2 achieves an increase in mid-lability carbon over just nine months. While there is fluctuation between three and nine months post-establishment, where some species show an increase between August 2021 and some a decrease, every combination/species has a net increase with no overlapping standard errors when the soil baseline is compared to the final sample. This indicates that while labile C is being assimilated by plants into biomass production, some of the labile C is being altered either by the plants themselves or by a microbial community related to the plants or reliant on plant exudates into a more recalcitrant form. This holds a positive implication for the use of herbal leys as transformers of labile carbon into more recalcitrant forms, even over short timescales. This must be contextualised in the frame of a total loss in carbon, however. There has been no net gain of C under any species or combination tested in WG2 over nine months, although there has been an increase C in the fraction burned off between 250-325^{o}C. Another important point to note in this discussion, however, is that not all of the processes turning labile to mid-lability C are plant-derived or controlled. As Fig. 4.13 illustrates, control plots saw the biggest initial increase in mid-lability C (although also with the largest SE) and also the largest drop between August 2021-February 2022. It is likely that these processes are microbially-driven, given no action by plant growth or rhizodeposition are happening, indicating that fallow soil can still maintain processes by which carbon changes pools even with increased vulnerability to weathering and erosion. Microbiota are main drivers of C change (Hartmann and Niklaus, 2012; Hirsch *et al.*, 2017; Matos *et al.*, 2019) and it is important to understand that these processes not only continue without plant mediation, but potentially the presence of plants reduces the fluctuation in carbon pools. However, if this allows greater retention of mid-lability C, such as the more modest increases seen in PCT, TP, TC, P and C compared to controls which go on to accumulate more mid-lability C rather than decreases (the greatest of which is observed in controls, but also observed to a lesser extent in CP and T), this may still provide a positive impact of herbal leys on more recalcitrant forms of C.

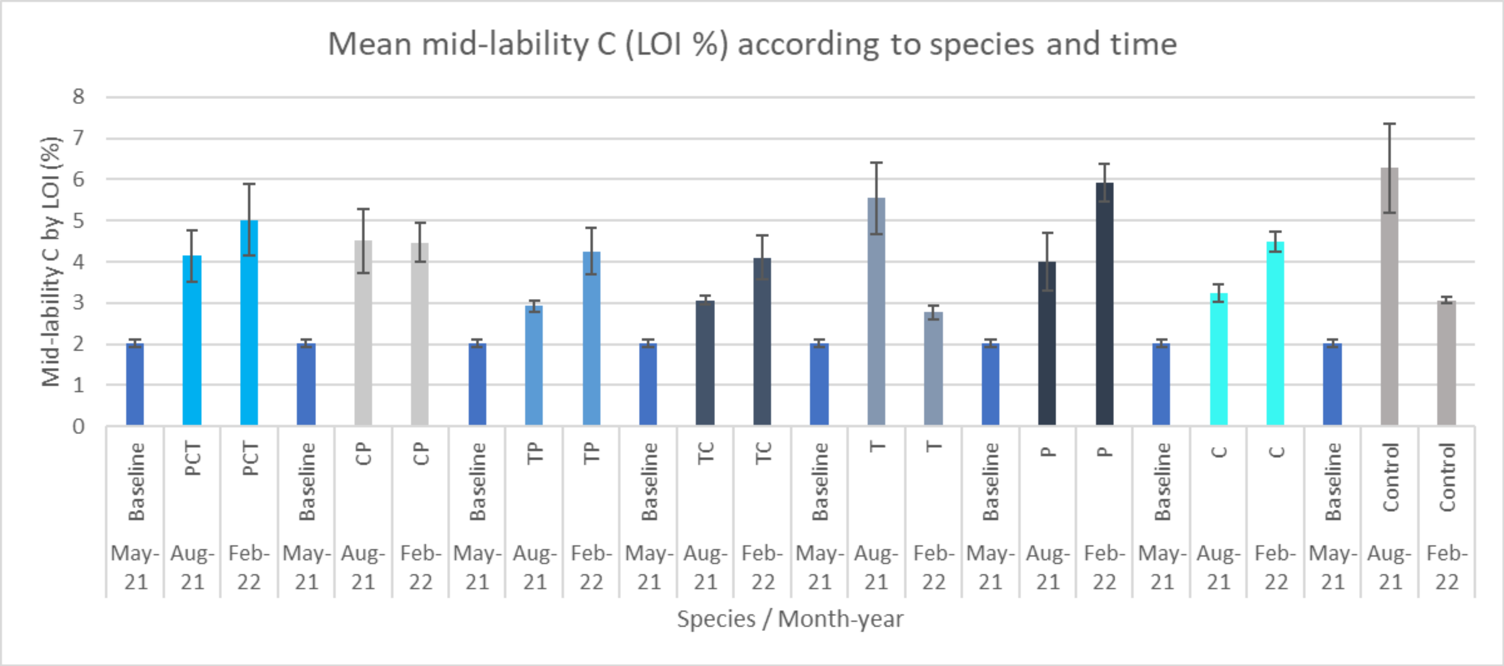


Figure 4.13 The progression of mid-lability C (LOI %) over time according to species, with baseline comparisons.

Depth

No relationship between mid-lability C and any depth interval was established with WG2. One possible explanation for this is that the temperature interval of 75°C is smaller than the 230°C to burn off labile C and the 175°C interval between mid and recalcitrant C, meaning less C lost in this interval. A smaller sample reduces data reliability and accuracy. Another reason is that mid-lability C is less different from labile and recalcitrant C than labile/recalcitrant are from each other at opposite ends of the spectrum, so it may be harder to draw a distinction between this form of C and a depth measurement. The other main explanation is likely to be time. A depth relationship was consistently observed in WG1, if only between the top 10 cm soil and the most labile C, but this is also likely limited by the short period of establishment (two years). WG2 was even more time-limited and therefore this is likely to be an even larger contributor to the lack of predicting power in the model. This is still an important piece of information for forming management and funding plans, in that leys in place for less than a year (and according to WG1, less than two years) are not likely to bring significant C benefits by increasing the proportion of C stored in less labile fractions.

Species

While almost all species/combinations were individually good predictors of labile C, the only species which drove change in mid-lability C was ribwort plantain. The consistent increase in mid-lability C under ribwort plantain is illustrated in Fig. 4.13, where this plant (P) has the third-highest increase in mid-lability C in the first three months, after the three-species combination (PCT) and timothy (T). Unlike T, PCT and P go on to see an increase in mid-lability C over the next six months, and P sees a greater increase than PCT. This could be related to rooting morphology, as ribwort plantain has a greater proportion of its roots at 60-70 cm and 70-80 cm depths than any other plant/combination apart from PCT (see Fig. 4.17 in section 4.4.6) and deeper roots are expected to influence soil C at greater depths by rhizodeposition. This is linked to the connection in the literature between depth and higher C recalcitrance (Lorenz and Lal, 2005). There are possibilities related to the compounds in ribwort plantain exudates, and their direct influence on soil C or their influence on the microbial community which synthesise C-containing compounds (Hodge, Grayston and Ord, 1996; Wurst *et al.*, 2010). This is evidence supporting the theory of a relationship between rooting depth and soil C by the medium of root exudates and subsoil tissues, with an increase in recalcitrance with deeper rooting to also support the theory of increasing recalcitrance with soil depth (Lorenz and Lal, 2005; Mellado-Vázquez *et al.*, 2016; Poirier, Roumet and Munson, 2018). This relationship between depth and recalcitrance was not, however, supported particularly well in WG1, where a link between shallowness and lability of C was established, but this was not accompanied by evidence for increasing depth correlating with increasing recalcitrance. This is likely related to the timescales on which WG1 and WG2 were established. However, this is an important fact to note because the experimental timescales are common timescales over which *in situ* leys are established. If an effect on C cannot be observed in one or two years in the mesocosms, they are not likely to have a positive effect on soil C in the field over much larger areas, which are also subject to more change in terms of wind erosion, weathering, tillage, artificial inputs, grazing or harvest, and legacy effects of degradation of soil structure and fertility.

That no other species significantly drove a response in mid-lability C is interesting. This could be due to a smaller window of opportunity for burning it off (a 75ºC interval rather than the larger intervals for labile and recalcitrant C). However, it could also be that other species or species mixes are simply not storing significantly more C in recalcitrant forms, especially over the short lifespan of the mesocosm. Similar rooting depths to ribwort plantain are observed in red clover, but the pattern between mid-lability C and red clover was not observed. Therefore it may not be the influence of rooting depth, but the exudates, life cycle, or influence on microbiota driving the difference in mid-lability C. This also indicates that legumes are not a significant driver of mid-lability C changes despite the well-established relationship between N and C (Stevenson and Cole, 1999; Rovira and Vallejo, 2000)

4.4.4 GLM_550

Basic soil parameters

No other variables are significant, including pH, BD, N, or nutrient content, which is more expected for recalcitrant C than labile. This is because the processes by which pH, BD or nutrients might be influenced generally take place over shorter timescales than recalcitrant C can be accumulated, and it would be difficult therefore to draw any parallels between them on such a short timeframe. There is also the evidence that increasing soil N increases labile C but decreases recalcitrant C, due to acidifying soil which reduces bacterial biomass (Jiang, Cao and Zhang, 2014). Considering this, it is likely that the processes in WG2 governing recalcitrant C accumulation have not reached equilibrium and the fluctuations are enough to mean any pattern is not discernibly significant in the GLM. The comparison between WG1 and WG2 indicates that N can start to become a significant predictor of recalcitrant C in as short a period as two years (see section 3.5.5). Therefore we can provide evidence that while one year is not sufficient to prove an N relationship, two years might be (depending on other factors, the other key difference between WG1 and WG2 being OM content). We can therefore draw the conclusion that while a C-rich soil may draw a benefit in increased N over two years, a C-poor soil is less likely to do so, and the relationship between C and N is weaker in C-poor soil.

However, more research is needed to separate the effects of increased C in the baseline soil from the time effect.

Time progression

No sample month showed as a significant predictor of recalcitrant C. There were identifiable patterns, illustrated in Fig. 4.14 but not enough for time to be an accurate predictor of C. After an initial drop in the first three months, PCT achieved a net gain of recalcitrant C of around 1.25% by weight lost on ignition. The only other treatment to also see an increase compared to the baseline level of recalcitrant C was TP, which achieved an almost 2% rise. All other species saw a drop in recalcitrant C compared to the baseline figure. The smallest decrease was seen in red clover, which also saw an increase over time compared to an initial drop, but still held a slight net loss in recalcitrant C by weight over nine months. Standard errors also show a lot of overlap between different species and sample months, which is reflected in the lack of predicting power in the GLM. Overall, only PCT and TP emerge as positive treatments likely to bring about an increase in the most recalcitrant forms of C over a period of less than a year, and the large SE in the TP treatment indicates that this is a less than definite effect, to be treated carefully. It has previously been discussed how the short period of WG2 (<1 year) will affect the outcomes of the GLMs and reduce their power in prediction, especially as processes linked to increased recalcitrancy of C are slow-moving and become more stable over time (Shi *et al.*, 2013; Wuest, 2014).

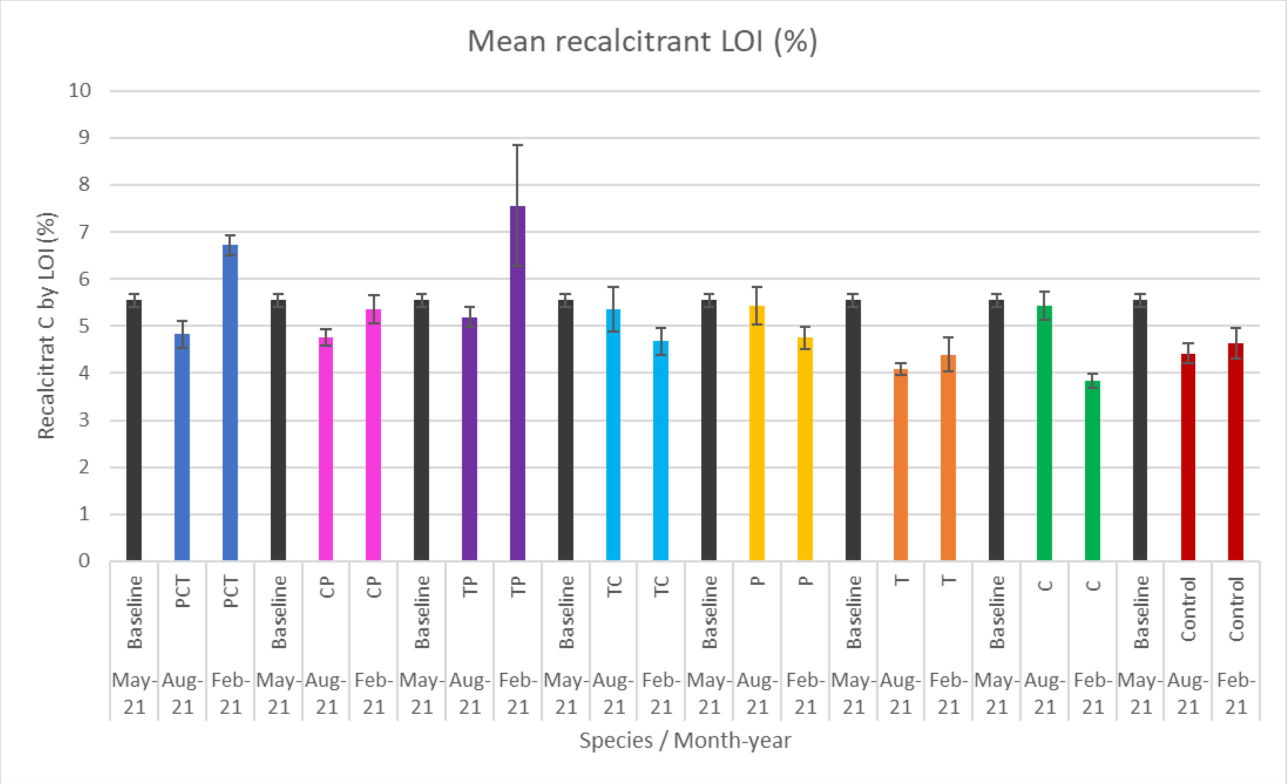


Figure 4.14. Comparisons of recalcitrant C by LOI (%) according to species and over time

Depth

No depths are significant in GLM_550, in line with GLM_250 and GLM_325. WG2 has provided no further evidence that a one-year ley can contribute to storing carbon in any particular depths, or that depth is related to C recalcitrance. In the literature there is some suggestion of recalcitrance being related to depth, as carbon is stored away from disturbance and oxygen, and there is a less abundant or less active microbial community keeping C in active forms (Fontaine *et al.*, 2007; Shi *et al.*, 2013; Jiang, Cao and Zhang, 2014). There is also evidence of the proportion of alkyl C increasing with depth, increasing the resistance of C compounds to decomposition (Lorenz *et al.*, 2007), as discussed in section 3.5.5. The lack of evidence for these processes in WG2 is most likely due to the short timescale of the experiment, but this is a relevant time frame to *in situ* leys, so this is an important point to note. It is likely that any processes which form recalcitrant C take much longer than the lifespan of the WG2 mesocosm, and that any processes by which C is drawn down or leaches through the soil to lower horizons also take much longer than the mesocosms, or leys in the field, are established for (Lorenz and Lal, 2005; Dodd *et al.*, 2011)

Species

The GLM modelling loss on ignition between 375-550°C produced a significant prediction of recalcitrant C from the timothy + ribwort plantain (TP) combination of species. This is interesting because the legume, red clover, is not involved in any significant recalcitrant C changes, where it might be expected that the addition of N from legumes might stimulate microbiota which are usually N-limited to synthesise more C-containing compounds from available subsoil OM (Fornara and Tilman, 2008; Dhamala *et al.*, 2017). Instead, the grass and herb combination has impacted recalcitrant C with a significant increase (Fig. 4.13). There is evidence than including herbs alongside grasses in leys is beneficial for soil C potential (Cooledge *et al.*, 2022), but legumes are expected to play an important role in any increase (Guan *et al.*, 2016). A potential explanation is the possibility that N-fixation in WG2 pots which incorporate a legume means the other plants are healthier, as plants ad especially grasses prefer N newly deposited by legumes to N already present in the soil (Dhamala *et al.*, 2017) and therefore contributing less to soil C by way of dead tissue (Phillips *et al.*, 2012).

4.4.5 GLM_LOI

Basic soil parameters

The total LOI could not be reliably predicted by any soil parameter. This is an interesting result because when C is split into fractions, we see several influencing factors. When the carbon is analysed as a whole, it could be that some of these factors interact, for example, the potential for increasing nitrate from OM humification moderating an increasing pH from other factors such as microbial exudates or the breakdown of any calcium carbonates present in the soil. Viewing the cumulative result of all the temperature intervals for total LOI loses the definition in C fractions which make significant variables clear. That means the biggest influence is in fraction of carbon, not total (which is a clarity on C that total C:N analysis wouldn’t provide). This tallies with the 250_GLM examining the most labile C in WG2 where the highest number of variables showed as strong predictors of C. This means that in WG2, the C differences are definitely being driven by changes in labile C and not mid- or highest-recalcitrancy fractions.

Depth

That no depths are significant across any fraction or total LOI indicates that the plants are not particularly influencing C at depth, for one or more reasons. It may be because it is their first growth year and they have not had time to root deeply enough, or influence the soil biotic and abiotic environment enough, in such a short amount of time (<1 year). This may in turn be influenced by the soil being considerably nutrient-poorer than WG1, and therefore a longer period is necessary to establish growth good enough to devote more resources to deep-rooting (Uteau *et al.*, 2013). The plants were noticeably smaller after their first year than WG1 plants were after one year, see Fig. 4.15 for comparison (noting that the most dominant plant on the right in the photograph of WG1 is chicory, which is not included in WG2).

Figure 4.15 Comparison of WG1 (top) and WG2 (bottom) 1 year PE

Species

Species did not show as significant in total LOI, despite high prediction strength across species and combinations for labile soil C; one species (P) as significant predictor for mid-lability C; and the TP combination as a significant predictor in the recalcitrant C model.

Figure 4.16 below illustrates the cumulative changes in total LOI (%) by fractions of lability, as a proxy for total C. Only PCT and TP show as having an overall increase in soil C by LOI (%) by the end of the experiment. Each species division has a reference to the baseline on the left. Standard error bars indicate there are significant differences between the fractions of C lost on ignition between species/combinations, but the GLM shows that these are too weak to act as good predictors. This could be as a result of limited time, which, as previously discussed, remains a relevant point due to the temporary nature of over 21% British grasslands (DEFRA, 2021b).

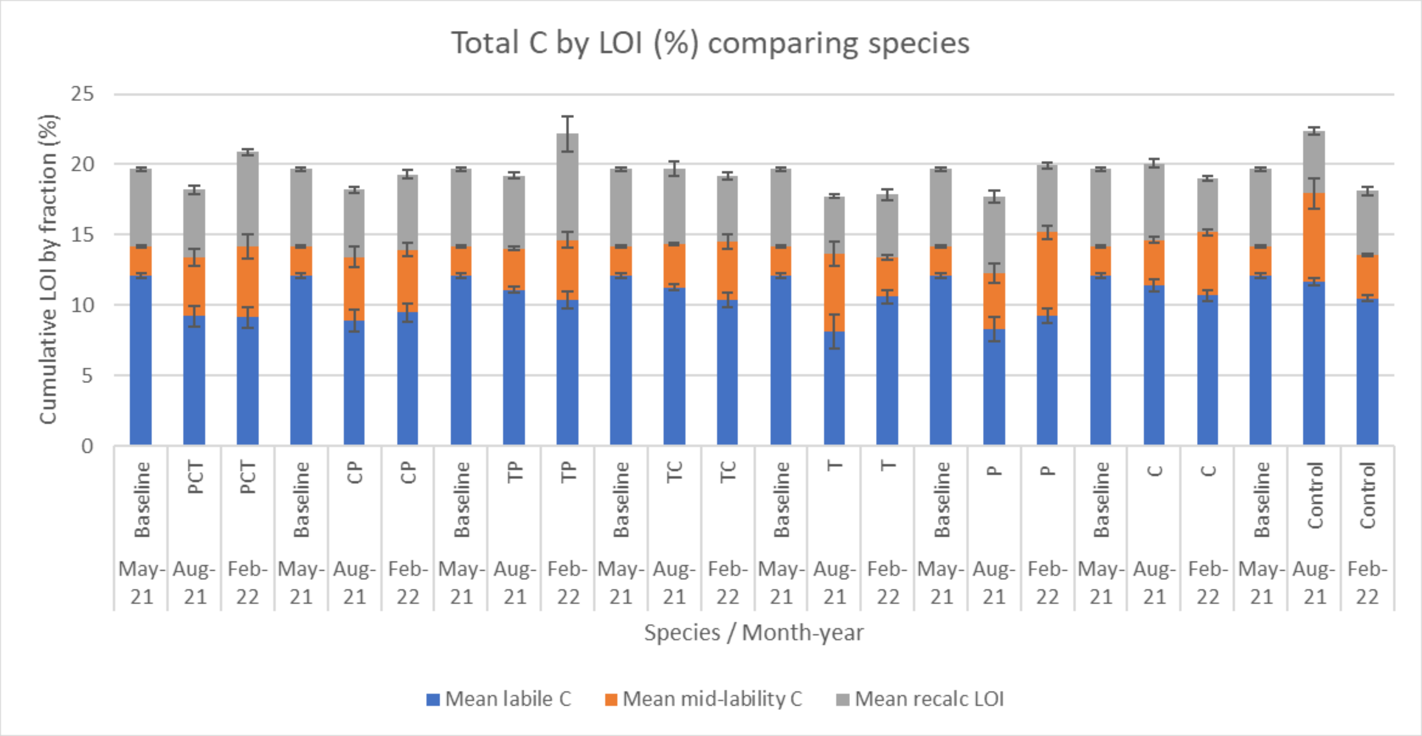


Figure 4.16 Total LOI by fractions over time, comparing species

4.4.6 Roots and C

Seven GLMs were run relating to root data. Predicting root biomass (g) was possible from labile and mid-recalcitrancy C, but not recalcitrant C or total LOI. Root biomass was also a good predictor of labile and mid-lability C, but not recalcitrant C or total LOI. As the influence of species and depth on C and fractions of C has been discussed above, and no conflicting evidence was produced in the root GLMs despite different interaction effects, only the root-depth, root-species, and root-carbon relationships are discussed in this section. Roots did, overall, have greater biomass in WG2 (poor soil) than WG1 (C-rich soil).

Depth was always a very strong predictor of root biomass (g) in every model across all interactions. This is expected, as there is a distinct pattern in root biomass with increasing depth, as shown below in Fig. 4.17, which shows the total root biomass, divided into depth intervals to give g 10 cm^{-1}. As Fig. 4.16 makes evident, the majority of root biomass is concentrated in the first 10 cm soil, although PCT mix has by far the most root biomass and the most biomass in the two lowest depth intervals (70-80 cm and 80+ cm). Timothy, as expected, has the most root biomass in the top 10 cm as a fibrous-rooting grass, whereas ribwort plantain has the least root biomass in the shallowest interval and the most evenly distributed biomass over the entire depth profile, although the second lowest biomass in total after red clover + ribwort plantain.

That red clover + plantain have a lower root biomass than ribwort plantain alone is unexpected, as clover is expected to support the growth of other plants (Dhamala *et al.*, 2017). However, a possible explanation for this result is that red clover is having a similar effect in plant mixes as was observed in WG1 with soil already rich in OM: that accompanying plants do not need as deep a rooting system because less biomass is required to imbibe the same amount of nutrients, when the soil is being enriched by a neighbouring legume. N is usually a limiting factor on plant growth (Hartmann and Niklaus, 2012) , and therefore it is a limitation of this study that aboveground biomass was not taken into account. It could be that less investment belowground in the company of a legume was producing a similar investment in biomass aboveground than plants which were not partnered with legumes and had larger belowground systems.

It is a positive result that the most diverse system, PCT, has both the heaviest and deepest rooting system (Fig. 4.17). PTC also produced a slight increase in total C by LOI and an increase in the proportion stored as recalcitrant C (Fig. 4.16). This is likely to mean that, given enough time, the rhizodeposition is likely to be greater at low depths with more species. This is a desirable outcome given that rhizodeposits are an injection of OM into horizons away from O_2 in air (reacting with C to make CO2, a GHG and common form of soil C loss) and from soil pores, which decrease in size and frequency with depth (Uteau *et al.*, 2013). This means breakdown is more likely to be anaerobic, and the products of biodegradation stored in horizons inaccessible to machinery disturbance and less accessible to further degradation by microbial action (Cotrufo *et al.*, 2013). Instead, humification will take place over much longer time periods by a smaller microbial community, slowly releasing C-compounds (Rovira and Vallejo, 2002), with residence time linked to recalcitrance (Das *et al.*, 2019).

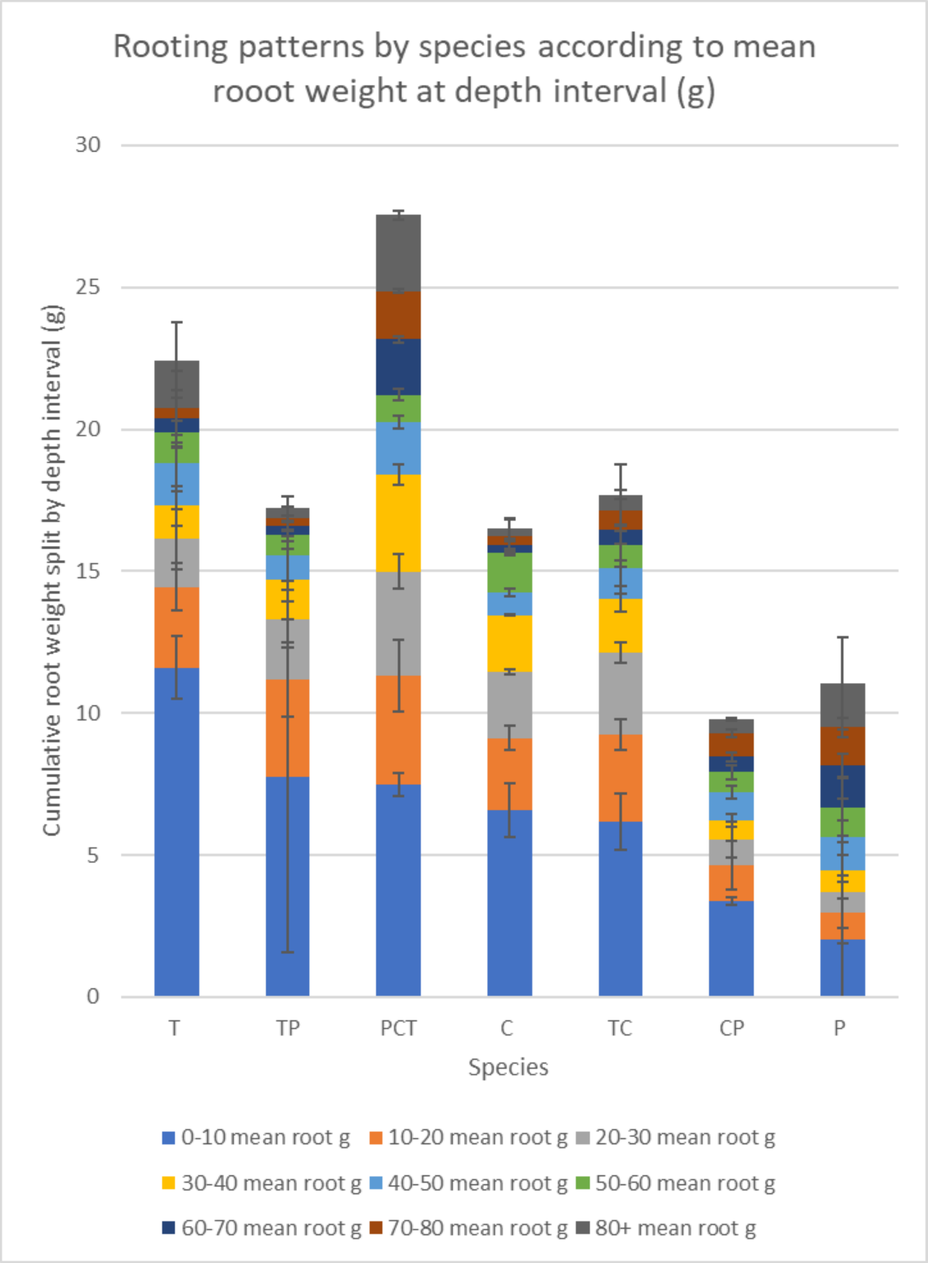


Figure 4.17 Total root biomass by 10 cm depth intervals, comparing species and combinations

Root biomass was not a significant predictor of total LOI (% weight loss) or recalcitrant carbon. This means that any change in carbon recalcitrancy as discussed in earlier sections cannot necessarily be attributed to greater (or less) root biomass. This indicates that there is a more important driver which has not been measured in WG2. The most likely answer to this is microbial biomass, the lack of data on which is a limitation of both the WG1 and WG2 mesocosms. It is established that microbial biomass has an impact on total and recalcitrant C (López-Mondéjar *et al.*, 2020; Elisabeth B. Ward *et al.*, 2021), and also that microbiota influence rooting structure and effectiveness, and provision some resources (Kaštovská *et al.*, 2015). This is a likely missing piece of information which would improve the prediction capabilities of the models.

Root biomass was, however, a significant predictor of both labile and mid-lability C. The pattern also works in the opposite direction: labile and mid-lability C are good predictors of root biomass, but root biomass does not respond to recalcitrant C and total C by LOI. Given the balance of root biomass tilting towards the top 10 cm of soil (Fig. 4.16), this does establish a link between labile and mid-lability C by means of root biomass, an indirect effect. This is an additional explanation of the patterns explored in sections 4.4.2 and 4.4.3 whereby depth influences labile and mid-lability C. Root biomass seems to contribute part of the explanation with a predicting effect of $p = 0.024$ and $p = 0.027$ in labile and mid-lability C respectively.

Species are not a significant predictor of biomass in any single or multi-species complex, which is an interesting result given the differences illustrated in Fig. 4.16. However, it is clear that there is a lot of variation within species, as much as between, given the overlapping SEs. It could simply be the case that the variation within species removes the possibility of significant species drivers of root biomass. With more time, more replicates could have been destroyed for a pool of >3 root replicates to increase the power of the analysis and reduce the SE. Time could also be a factor as WG2 was destroyed after one full year of growth, whereas WG2 had over two years of growth, which is important when ribwort plantain (both experiments) and chicory and dandelion (WG1) live for two or more years. However, the results observed after one year are still valuable given the short lifespans of herbal leys, and species cannot be relied upon in one year in poor-C soil to produce a consistent rooting depth or effect on C related to depth in more recalcitrant forms, despite patterns emerging which indicate a positive trend in that direction (Fig. 4.16).

4.5 Conclusion

4.5.1 Soil parameters

WG2 soil had a finer texture and higher clay content than WG1, which indicates a reduced OM load. Particle size and higher clay content increase SOC storage potential when OM is introduced.

BD may have had accuracy issues, addressed in the discussion and below in the limitations of the study. Resampling interference may have implications for other variables including C. BD was not a predictor of labile, mid-lability, recalcitrant or total C, although it interacts with many other variables to have both direct and indirect effects on soil C including plant rooting and water transport. This may be the result of a short time frame which is not enough for BD to prove an effect, or it could be that BD is not a limiting factor in any C-related processes in WG2.

Water was not a predictor of labile C, despite water mediating several processes which affect C including microbial activity, plant growth and nutrient transport. Water may have not been a limiting factor on the plants in WG2, and time is also a potential mediator in the effect of water transport and leaching because of the short duration of the experiment. However, water is a significant predictor of mid-lability C, which may be an alignment between water content and a greater demand for it in the processes which dictate mineralisation of more recalcitrant C.

Labile C and mid-lability C are not responsive to pH, which may have been due to a short timescale insufficient for a detailed account of changes in N and P compounds which influence pH. The processes driving pH change generally take place on longer timescales than those which influence labile C so this is not necessarily unexpected.

Nitrite, nitrate and phosphate had no influence on labile C, despite established P-C and N-C relationships described in the literature. These variables may have an interacting effect but likely the timescale is not long enough to observe significant changes or establish a relationship between them and C. Phosphate did, however, have an effect on mid-lability C, which may be due to similar microbial communities or processes by these which govern the production of both phosphate and more recalcitrant C. Nitrate and nitrite were not predictors of mid-lability C, indicating that these N compounds may be transitive between labile and recalcitrant and either do not persist where soil C is in a state of medium lability, or that the time frame of the experiment was again too short to detect this level of detail.

No parameters influenced recalcitrant C. It is likely that the processes in WG2 governing recalcitrant C accumulation have not reached equilibrium in the nine-month lifespan of the mesocosm, and the fluctuations are enough to mean any pattern is not discernibly significant.

No general parameters showed a predicting effect on total LOI, despite more distinction when C is split into fractions. This may be because different processes influencing different recalcitrance pools are mediating each other. The models provide evidence that changes in total LOI are driven by changes in labile C.

4.5.2 Species

That all species showed C fluctuations, but controls also showed reductions in soil C, provides evidence that not all C fluctuations are plant-derived but up to 2% the soil C is activated by other processes, probably microbial. However, plants are responsible for some of the C decrease as C is incorporated into plant biomass. Species are significantly different in their influence on labile C, which provides evidence of a benefit in encouraging biodiversity in leys. Given that the period of time over which ley experiments are usually established is much longer than WG2, it is likely that species effects are diluted here by short timeframes. While all species/combinations drive change in labile C, only ribwort plantain is a strong predictor of mid-lability C, potentially related to rooting morphology, symbionts or exudates.

Species is not a driver of mid-lability, evidencing that most C change is driven by change in the most labile fraction. However, timothy + ribwort plantain does have a significant predicting power for the most recalcitrant fraction. Legumes are not influencing recalcitrant C accumulation over <1 year. This potentially is related to increased plant health and decreased senescence in pots which include a legume.

Species do not drive total LOI despite high prediction strength in the labile fraction, the main driver of change, and several species predictors evidenced in mid-lability and recalcitrant C. There may be significant differences between the fractions lost on ignition between species, but they are too weak to act as good predictors, possibly as a result of limited time.

Diversity does, however, improve soil C content, with both PCT and TP treatments producing a slight increase in overall LOI (%).

4.5.3 Depth

Depth is not a predictor of any fraction of C. WG2 does not add to the evidence in the body of literature around lability and shallowness, or recalcitrance and depth. Time is likely to bring definition to the patterns which are starting to emerge. Timothy + ribwort plantain, and timothy +

red clover combinations produced the effect of storing an increased proportion of soil C at greater depths, although still produced a net loss of soil C. The lack of evidence for increasing proportions of recalcitrance in WG2 is likely due to the short establishment period, which is also important to consider given usual ley lifespans *in situ*. That no depths are good predictors of total LOI also indicates a potential time effect, as plants which have several years' growth left will not have exercised their full influence on the soil in terms of growth and senescence cycles of increasing and decreasing rhizodeposition, and belowground biomass production.

4.5.4 Time

WG2 was established for one year, when it was destroyed for roots. Soil samples were taken three and nine months PE, which is a short time even by temporary ley standards. However, time still showed a significant influence on labile C, which showed seasonal fluctuations due to plant senescence and growth patterns.

Time progression was significant only for changes in labile and mid-lability C, and not total C or the most recalcitrant fraction. This indicates that a year is not enough to observe C changes which may be possible in a C-depleted soil under a herbal ley. However, it is an important result that no species achieved a net gain in soil C over this time, which can feed into management plans and policy. Despite no net increase, the evidence showing a greater proportion of C was transferred to the mid-lability pool over the duration of the experiment is a potential benefit for reducing soil C bioactivity and storing it in fractions more robust to land use or temperature change. There are changes in the proportions of C stored in different pools of recalcitrance over time, including the least reactive pool, which is a positive indication that moving C into more recalcitrant pools through the action of a diverse ley is possible, even if it needs more time.

Given that time shows only in the most labile C fraction as a strong predictor, it is likely that changes in total soil C are driven mostly by changes in the labile fraction. Microbial mediators are also likely to be active drivers of changes over time, as mineralisation processes by microbiota run on different time scales according to abundance, diversity, the nature of OM inputs, and the recalcitrance produced from these.

4.5.5 Roots

Root biomass is a predictor of labile and mid-lability C, but not recalcitrant or total C. Root biomass has a strong relationship to depth at every depth interval tested and most root biomass is concentrated in the top 10 cm soil. From these two facts, it can be inferred that different root biomasses at different depths are driving an effect in labile C, indirectly linking depth to C lability, even though the pattern was too weak to be detected in the GLMs. That the most diverse treatment (PCT) also had the heaviest root biomass and more of it at depth also draws a positive relationship between diversity and root biomass, which influences rhizodeposition, which could potentially be used to infer a relationship between depth and soil C on a longer timescale. However, the evidence of WG2 does not prove any change in soil C recalcitrancy can be attributed to root biomass.

4.5.6 Management implications

That no species or combination achieved a net gain in SOC over the nine-month period is important information. This should inform policy which subsidises one-year leys, given the approach to

Environmental Land Management Schemes (ELMS) being brought in in the UK from 2023 which include an incentive for improving soil heath. The WG2 mesocosm shows that one year is not enough time for herbal ley growth alone to improve soil health. It may have benefits alongside other management strategies, but time is likely to be a key player in the effectiveness of encouraging plant biodiversity for its C-storage effects.

It is also the case that solely soil-derived C benefit is not the total benefit from herbal ley cover cropping. Increased soil C by the action of plants (on a longer timescale than WG2) and potentially incorporating this as green manure which would involve sacrificing a hay harvest would also mean reduced fertiliser inputs, which come with their own carbon cost in terms of production and transport.

4.5.7 Limitations

Measuring BD may have caused issues with accuracy relating to resampling interference, introduction of novel bacteria or O_2. These have implications for the accuracy of other parameters including C.

Plant growth was not directly measured, which is recommended in similar future studies to compare growth patterns in different media and to assess the partitioning of resources between above- and below-ground biomass.

The short time frame of the experiment (<1 year) is likely a limiting factor on many variables showing insignificance. Given time, more clarity may emerge in the patterns. However, this is not a failing of the experiment given the short lifespans of many leys in the UK, which are usually established for 1-5 years.

The lack of clarity on microbial abundance or biomass is a limitation of the study given how important microbiota are in C-synthesising processes.

4.5.8 Suggested future research

Future research should incorporate an aspect of microbial community, by diversity, respiration, abundance or biomass, given the important mediating effect soil microbiota has on many processes directly and indirectly influencing soil C. Gaps where patterns are indistinct, unexplained or simply visible but insignificant may be explained in a model where a measure of microbial activity is a potential predicting factor.

Also missing from WG2 was an aspect of how plants partition resources to above- and below-ground biomass, given the results in root biomass showing unexpected effects when herbs and grasses are accompanied by legumes. Future studies should incorporate some periodic and non-destructive measure of plant growth to better inform models of resource utilisation in plants.

Future research should also incorporate a secondary management post-ley establishment, such as turning a cover crop to green manure. It is evident that cover cropping for one year is unlikely to bring a C increase, but plants synthesise C from the atmosphere as well as the soil. Physically incorporating biomass into the soil is likely to provide a much greater impact on soil C, given the evidence for OM inputs improving soil health.

5: Grassland plant-mediated effects observed in situ in grass-dominated plots in a grazed pasture in Leicestershire

Abstract

Current land management schemes prioritise short-term productivity over long-term sustainability. Processes influencing soil organic carbon (SOC) change can be species- or cultivar-mediated. Manipulating the species or cultivar diversity of a ley may bring long-term benefit, either through increases to total SOC, transferring carbon (C) from labile to recalcitrant fractions, or by sequestering C deeper below ground and reducing C vulnerability to environmental change. Temporary leys comprise over 20% the UK’s grasslands and could be an important tool in reducing soil greenhouse gas (GHG) emissions, bringing improvements in both soil health and reaching climate targets.

The soil supporting three replicates of a Broadsword ryegrass control and five Festulolium cultivars was analysed for differences in basic soil parameters (pH, water (%), bulk density) as well as differences in C influenced by depth and root biomass.

Hypotheses:

1. Cultivars show differences in the C content of supporting soils
2. Differences in SOC are driven by differences in root biomass
3. Deeper rooting cultivars will store a greater proportion of their C in more recalcitrant forms
4. Shallow rooting cultivars will store a greater proportion of their C in more labile forms

Cultivar was a powerful predictor of labile, mid-lability and total C. Depth was a significant predictor of labile, mid-lability, recalcitrant, and total C. Root biomass was never a significant predictor of any form of C. No other soil parameters had a significant influence on total C or fractions of C. Because of the lack of influence of root biomass, the cultivar-driven subsoil changes in C are being mediated by another variable. It is suggested that this is likely microbial activity. Observable patterns between root biomass and C fractions were not significantly different between depths or cultivars, although some indications emerge that Fojtan is the likeliest of the cultivars to store C in more recalcitrant fractions; Aberniche the likeliest of the analysed cultivars to store the most C at depth; and Broadsword or Lofa the likeliest to transfer labile C to more recalcitrant fractions.

Overall, the greatest evidence provided from this experiment is that diversity is an influence on increasing SOC stock. Future research should incorporate more timepoints and a microbial element to the experiment. Information on legacy effects would also be useful in developing management strategies around ley establishment.

5.1 Introduction to Leicestershire experiment

The application of research to real in-situ landscapes requires testing by real-life scenarios, and in reality, very little land is simply left to develop without interference; instead, under the current land management schemes, agricultural productivity is prioritised and even fallow areas are part of a wider landscape under a range of pressures, and are often incorporated with dual purpose into the land management of the catchment (Virto *et al.*, 2015).

For this reason, it is important to test the carbon storage efficacy of these carefully selected, incentivised plants, under the incentivised conditions under which they are expected to grow. While this includes the biochemical management of the soil such as the addition of fertiliser, herbicide or even water, this can also include physical expectations such as hay cutting or direct grazing to support livestock. The legacy conditions of the soil, such as the length of time it has been farmed, its position in a regular rotation, compaction by machinery, and historical alternative uses such as settlement or waste disposal, will all hold influence over the outcomes of the application of research aiming to increase soil carbon.

5.1.1 Historical land use

Land use change, and its acceleration over the last century, has compromised the ability of current manipulated and exploited ecosystems to sustainably meet the needs of human wellbeing and those of the occupying biodiversity (Jiang et al., 2013). Land use change is a major driving factor in SOC stocks and thereby also has a bearing on atmospheric carbon concentrations, which is of most pressing environmental and legal concern (Poeplau et al., 2011). Land use change is the second-largest source of human-induced GHG emissions after fossil fuel combustion (IPCC, 2007). However, losses directly from soils are generally greatly underestimated as a contributor to either atmospheric CO_2, or their C storage potential (Poeplau et al., 2011). Natural vegetation cover across the world has greatly decreased due to human activity, especially since the 1950s, mainly due to demands for agricultural land leading to conversion of primary forest (Poeplau et al., 2011). Land use change alters C inputs and decomposition rates, alteration of the rate of harvesting of net primary productivity and thereby natural returns of C to the soil, and alter the physical parameters of the soil such as aeration, moisture and temperature which influence the structure and chemical processing (Poeplau et al., 2011).

SOC is not an isolated process, and C sequestration is reliant on multiple ecosystem services (ES) and the intricacies of interdependent webs of biodiversity (Jiang et al., 2013). Stabilisation of processes within soil are also assumed to take around 20 years on average and even longer in temperate regions (Poeplau et al., 2011), and therefore data taken immediately after conversion is unlikely to meet a true baseline, whereas much longer-scale mapping on decades of data could miss important fluctuations.

At the landscape scale, the interdependencies within ecosystems, including those on which SOC storage is reliant, are so complex that predictions to ecosystem services become very difficult, and this create many challenges in terms of delivering policies and responses to declines in ES (Jiang et al., 2013). Historic land use change with regards to soil carbon tends to focus on net area difference between two time points (Jiang et al., 2013), whereas an in-depth accounting for all changes over a given time frame would give a much more accurate assessment of the fluctuations in accordance with the land use (Fuchs et al., 2015). With regards to a specific farm or even field, the historical land use data has to be incredibly precise to model long-term changes to soil carbon, and this precision is often absent. Land is simply assigned broad categories of vegetation cover (Fuchs et al., 2015). Many land-use change models address an entire country or even most of a continent, which lacks the fine-scale detail necessary to address soil carbon on an individual field, farm or catchment (Soussana *et al.*, 2019). Indeed, the UK has been subject to a great deal of change between 1950-2010; so much so that it was excluded from a final pan-Europe meta-analysis as, according to official data, UK rates of change were >10% year^{-1} in forest and >15% year^{-1} in cropland, compared to respective European averages of 1.2% and 3.3% (Fuchs et al., 2015).

While the UK has good historical databases of land cover and use for at least the past century, increasingly data has to be extrapolated from what little information is available and therefore reliability and precision are compromised. Focusing on one small area or one reduced timeframe is a way around this issue (Jiang et al., 2013), but conducting this process for every farm or even river catchment would be an almost impossible task; therefore, a compromise between scale and accuracy must be met to most efficiently make recommendations for management on parcels of agricultural land, which meet the likely legacies of the soil on which they sit.

In order to most efficiently catalyse natural processes into effective carbon storage, first the baseline has to be understood; for example, if a soil has the capacity to return to a peaty scrubland without human interference such as drainage, because that was its original form, turning it into a grassland or herbal ley is not going to be the most effective way forward in returning the soil to a carbon sink. On the other hand, if a degraded but greened piece of land has previously been the site of waste disposal such as slag heaps, initiating a planting and maintenance regimen beginning with a combination of grass and herbs to allow natural succession would be far more effective than allowing it to lie empty, and could be a multipurpose solution including reducing visual pollution, providing grazing, stabilising mounds, and acting as carbon storage.

5.2.2 Grasslands in traditional agriculture

The UK saw its greatest land conversion rate in the 1940s (Jiang et al., 2013). The greatest SOC flux to the atmosphere up until 2007, and therefore likely up until the present day given recent relative agricultural stability, occurred in 1942 during the ‘Dig for Victory’ campaign to convert all available land, including gardens and parkland, to food production (Bell et al., 2011). Subsequent land use change has been mainly from semi-improved grasslands used for pasture to intensive crop-based agriculture (Jiang et al., 2013). Dorset, a rural county with excellent data availability for the 1930s and 40s, is likely representative of UK agriculture whereby improved grasslands were the single largest contributor to the increase in Dorset agricultural productivity between the 1930s and 2000 (Jiang et al., 2013).

There was a fall of around 2 million tonnes of carbon in the total SOC stocks of Dorset between 1930-2000, the majority of which was lost from the semi-natural habitats, particularly unimproved grasslands which were converted during that period to arable land or improved grassland (Jiang et al., 2013). Carbon became more focused in ‘hotspots’ created due to fragmentation of habitat (Jiang et al., 2013), and concentrated under some vegetation types and managements (Medina-Roldán, Paz-Ferreiro and Bardgett, 2012).

5.2.3 Grassland rotation

The conversion of cropland to grassland shows a continuous accumulation of C when the grassland is left in place; the Poeplau et al. (2011) study modelled sites with a minimum of 100 years establishment of grassland from cropland, predicting 40±11% SOC after 20 years and 128±23% after 100 years, the highest gain of any of the modelled land conversions in this study, greater than cropland to forest (16±7% after 20 years, and 83±39% after 100 years).

The type, management and exploitation of the grassland does have a significant impact on its C storage capacity; unmanaged grasslands accumulate the most C and pastures and meadows used for forage production showed significantly less C accumulation over the same period (Poeplau et al., 2011).

The good data availability in England and Wales has led studies to suggest that SOC losses to the atmosphere are increasing where land use remains in constant arable use (Bellamy et al., 2005; Smith et al., 2007). This is in contrast to the year of greatest national SOC sequestration, 1993, the year that the scheme of set-aside land in agriculture was brought in (Bell et al., 2011). However, there are some disagreements about the state and storage rate of SOC. Bell et al. (2011) modelled the UK soils from 1925-2007 and, with large fluctuations between loss and gain in the meantime, have found that on average the nation has seen a 5% rise in SOC levels since 1925. Accepting that their data is not flawless, because data is not available for every year and every county, let alone field, this is still a positive suggestion that the contribution of grasslands can make a difference to SOC on a national scale.

While the timescale of the Bell et al. (2011) is too coarse to consider the temporary grasslands in an agricultural rotation, given the fact that a certain area is likely to be maintained as grassland at any one given time – fragmented and rotated, but a steady acreage – the presence of such is still likely to hold a positive impact for soil carbon on a national scale. The biggest challenge, therefore, is establishing the short-term losses and gains from a real grazing scenario and establishing whether these are small fluctuations on a long timeframe or whether they can, as a whole, contribute to sequestering atmospheric carbon despite the land coming back into arable service.

5.2.4 Grazing: inputs and impacts

British blanket bog, 95% of which is grazed or used for grouse shooting, often accompanied by drainage which degrades the peat and reduces the peat-forming sphagnum moss, thereby degrading a major carbon sink (Natural England, 2009). Heathland, traditionally used to graze common livestock but now frequently privately owned and managed for restricted leisure activities (Rotherham, 2008), has been lost in conversion to woodland by 4000 ha since 1930 (Jiang et al., 2013). However, since the capacity to store carbon is much greater beneath woodlands than heathlands, conservation efforts may be hampered by ‘shifting baseline syndrome’ (where perception of a more recent semi-natural state biases efforts to return to a natural state (Guerrero-Gatica, Aliste and Simonetti, 2019) where heath is perceived as the best case scenario. However, these effects must be balanced in consideration with others, as tree planting does not only affect soil carbon, but also water availability, flood potential, biodiversity, and soil quality (Jackson et al., 2005; Jiang et al., 2013).

An important consideration for attempting to make a carbon-negative grassland, which stores more C than it emits, is its role as pasture for livestock. Agriculture accounts for 44% UK emissions, to which livestock enteric emissions contribute 70% (DECC, 2014). Grasslands which are left alone to grow and decompose are known to have a high carbon storage rate (Fuchs et al., 2015; Liu et al., 2017; Poeplau et al., 2011). But if this biomass productivity is not returned to the soil, and is consumed on-site by livestock, the chemical contents and the processes of returning them to the soil are changed by the acts of grazing and digestion, in addition to the direct contributions of livestock to GHG emissions.

Balancing this localised carbon budget requires knowledge of direct SOC flux and SOC storage, as well as the livestock emissions. In Canadian cattle-grazed grass-only (no herbs) pasture, McGinn et al. (2014) quantified the CH_4 and CO_2 exchange from both soil and cattle, across daily and seasonal variation. The study found an Aberdeen Angus heifer reached on average 189 g CH_4 animal^{-1} day^{-1} enteric emissions and CO_2 emissions of 4200 g animal^{-1} day^{-1}, with one pasture feeding 40 heifers for a day; the single pasture responsible for the 40 animals for one day reached a daily average of 8.64 g m^{-2} day^{-1}, indicating the grassland surface was a source of CO_2 rather than a sink. The peak in CO_2

exchange coincided with the period of peak grass growth, producing a sinkage of 52.8 g m^{-2} day^{-1}, but CO_2 exchange declined to 0 mid-August and was in the negative for the majority of the year. Emissions fluctuated from the grassland over the day, reaching its peak exchange around midday, the flux coinciding with the increasing and then decreasing solar radiation, the relationship losing distinction in the afternoon with high evaporation leading to low soil water availability, limiting CO_2 uptake by the plants (McGinn et al., 2014). This study also established that under a cattle stocking density of 0.1 or 0.2 beef heifers ha^{-1} (5 ha^{-1} per animal, or 0.16 livestock units (LSU) ha^{-1}), the grazed grassland achieved a small sinkage of 40 kg C ha^{-1} yr^{-1}.

In terms of sheep grazing, emissions are lower than cattle production but still significant enough to need an effective GHG mitigation plan in any nation producing their meat and/or wool on an industrial scale (A. K. Jones et al., 2014). While the enteric emissions of any livestock is likely to depend on the species and breed, and what type of pasture they are grazing (Fraser et al., 2015), there is limited information on this for sheep. Sheep meat is only 10% that produced in the UK but accounts for 16% the GHG emitted from UK livestock (DECC, 2014). Using CH_4 chambers and gas analysers, Fraser et al. (2015) established that pasture type had a significant effect on daily CH_4 emissions, which was higher on a ryegrass diet than the more diverse permanent pasture. The dual focus of Jones et al. (2014) on N_2O and CO_2 as GHG gases, rather than concentrating on flux from specifically carbon-containing compounds, also misses the potential carbon loss in CH_4 emissions in the integrated livestock pasture. While the public are undereducated about farming impacts on the environment generally (Padilha et al., 2021; Ricart et al., 2018; Vergunst & Savulescu, 2017), sheep seem tend to be perceived by the public as substantially less environmentally damaging than cattle (Lane, 2018; R. Liu, 2021), although available information on perception is very limited; however, the CO_2-equivalent calculation showed an average 100-year global warming potential of 11.86 kg CO_2-equivalent per kilo of liveweight in sheep, falling only slightly short of the 12.65 kg CO_2-equivalent for cattle (AHDB, 2012). Sheep also eat more than cattle per kg of their bodyweight (Lane, 2018), browse selectively and intensely resulting in lower SOC concentrations and reduced rates of C mineralisation (Barger et al., 2004), and are more frequently grazed on upland soils in the UK which are perceived as good for nothing else, causing great ecological damage to those plant communities and soils even on short (<10 year) timescales (Barger et al., 2004; Evans, 1997; Medina-Roldán et al., 2012; Meyles et al., 2006).

According to Natural England (Natural England, 2009; Rank & Spedding, 2009) 2.4 million ha of lowland, and 2.2 million Less Favoured Area (LFA) uplands, are used for livestock grazing in the UK. Permanent grassland accounts for 41% the total English utilised agricultural area (DEFRA, 2020a). In the uplands, 12,069 ha are overgrazed compared to 607 undergrazed (conservation grazing can be an important tool for maintaining species, sward height and microhabitat diversities), and 22,042 ha of blanket bog is overgrazed compared to 765 undergrazed (Natural England, 2009). The average stocking density over all lowland forage area in the UK is 0.58 LSU ha^{-1} (Rank & Spedding, 2009), equivalent to around one medium-sized bull of one-two years old, or around six medium-sized ewes (Nix, 2004). In June 2020, the main grazers of utilised agricultural areas numbered 5.2 million cattle and calves and 15 million sheep and lambs in England alone; 41 % of the English utilised agricultural area of 9.02 million hectares (DEFRA, 2020a) leaves 3.7 million permanently grazed ha of grassland just in England.

Calculating stocking by ha according to the DEFRA (2020) report on farming statistics, assuming that calves make up 60% the cattle population as breeding females constitute 35%, using Nix (2004) definitions of LSU:

Table 5.1. Land requirements at current recommended LSU and SOC-positive LSU rates

Animal	LSU ha^{-1}	Number of individuals in England (millions)	Land required at current LSU by population (ha)	Land required (ha) to maintain <0.16 LSU or less
Sheep	0.1	7	700,000	700,000
Lamb	0.04	7.7	308,000	308,000
Cattle	1.0	1.82	1.82 million	9.1 million
Calves (less than 1yr)	0.4	3.38	1.35 million	3.375 million
Total	N/A	19.9	4.17 million	13.48 million

The land requirements to maintain a SOC-positive grazing LSU in Table 5.1 are likely an underestimate, given that not all calves are under 1 year old, and calves at 1-2 years old have LSU of 0.7. However, this is an approximation using one source of data for LSU (Nix, 2004) and only one source of data for an LSU-based SOC-positive approach (McGinn et al., 2014).

England is 13.29 million ha (Office for National Statistics, 2020); therefore, the above recommended LSU required to graze the current English population of livestock at the rate recommended by McGinn et al. (2014), 13.48 million ha, is more than the entire land area of England.

Calculating an approximate carbon-sustainable LSU for our livestock populations according to the Natural England (2009) and Rank & Spedding (2009) data on area of grazed grasslands, and the Nix (2004) LSU values, the 4.6 million ha grazing land could support 736,000 cattle OR 7.36 million sheep in a carbon-sustainable way according to McGinn et al. (2014).

The concept addressed here has been taken through a much more detailed information-gathering process and in-depth analysis in Chapter 6 of this thesis. I thought the concept of SOC-positive grazing in the frame of grasslands, which is, after all, the purpose of most of them in the UK, could provide a fascinating insight into part of the agri-industry which is under-researched. This lesser-known emissions source is salient for informing the policy- and decision-making processes necessary to future-proof the British farming industry (and, indeed, the whole country) by identifying routes to GHG emissions, which then can be designed out, mitigated, or legislated against.

5.2.5 Methods in practice in grazed grasslands to increase SOC content

Currently, grazed grasslands do store some carbon, but on average, less than grasslands which are managed by cutting (J. F. Soussana et al., 2007). The highest C sink activity across the nine sites studied by Soussana et al. (2007) was indeed a grazed site, with a sink of 464 g C m^{-2} in the first year, but the lowest sink activity of 49 g C m^{-2} in the second year of observation was also in a grazed site. Grazed sites also show greater yearly variability in their annual net ecosystem exchange of C than cut grasslands, and emission vary with stocking density (J. F. Soussana et al., 2007). Besides the baseline of maintaining grassland, the carbon storage capacity can be enhanced with improved grazing management, concentrations of forage species, fertiliser application, irrigation and restoration (Mahanta et al., 2020).

A grazed field in Halstead, Leicestershire, established by the Game and Wildlife Conservation Trust (GWCT) is aiming to establish differences in soil health over several years and several treatments. This chapter focuses on research conducted in October 2021. Grazed for almost three years and established on soil previously supporting a homogenous ley treated with artificial inputs, the C balance of new potential cultivars was investigated.

The Halstead experiment incorporates several factors which were not discussed in Chapters 3 and 4 of this thesis, including grazing; diversity only in the form of different grass cultivars, rather than a herbal element; prior inorganic treatment, which may serve a legacy effect; and the fact that the ley is established on a working farm rather than as an ex situ mesocosm, open to much more environmental variability and mechanical intervention. It is important to incorporate these elements as a mesocosm is not representative of the effects of farming soil for decades, as Halstead is, and on such a wide scale with interacting variables including the presence of animals.

The hypotheses of this experiment are therefore:

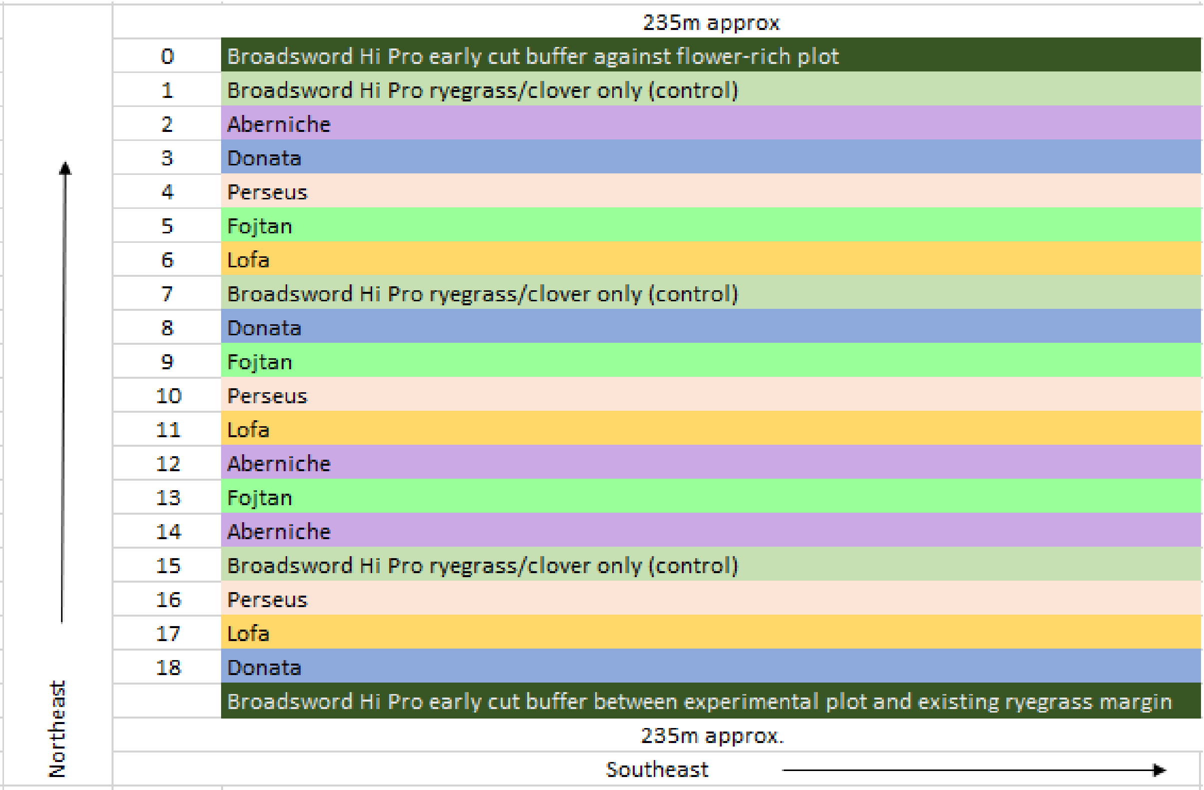
- Cultivars show differences in the C content of supporting soils
- Differences in SOC are driven by differences in root biomass
- Deeper rooting cultivars will store a greater proportion of their C in more recalcitrant forms
- Shallow rooting cultivars will store a greater proportion of their C in more labile forms

5.3 Methods

5.3.1 Planting regime and field methods

The experimental grass ley was established on a compacted clay soil in 2016, (52°38′51″N , 000°52′57″W) at Halstead, Leicestershire. The plots were grazed in their entirety for nearly three years. In early 2019, fences were established down the centreline of the plots, allowing one side to be grazed one side of the plots to be ungrazed for one year. Pits were dug in the plots to 70 cm depth by GWCT in 2019, therefore the soil had had some disturbance prior to sampling.

The layout of the experimental ley was three replicates of five test cultivars, buffered each side by a ‘Broadsword Hi Pro’ cultivar which was established at the field edge against a flower-rich margin. Three control replicates of ‘Broadsword Hi Pro’ with 5% clover were laid in strips as well as the test cultivars. The other grass strips were Aberniche, Donata, Perseus, Fojtan and Lofa. Donata is a cocksfoot grass cultivar and the four others are Festulolium cultivars. All are calorie-rich grasses bred to graze livestock, with different rooting depths and drought tolerances. The 18 cultivar strips, buffered against the flower margins with Broadsword, were laid the length of the field and randomly allocated with three replicates of each. Fig. 5.1 below illustrates the layout.



	235m approx
0	Broadsword Hi Pro early cut buffer against flower-rich plot
1	Broadsword Hi Pro ryegrass/clover only (control)
2	Aberniche
3	Donata
4	Perseus
5	Fojtan
6	Lofa
7	Broadsword Hi Pro ryegrass/clover only (control)
8	Donata
9	Fojtan
10	Perseus
11	Lofa
12	Aberniche
13	Fojtan
14	Aberniche
15	Broadsword Hi Pro ryegrass/clover only (control)
16	Perseus
17	Lofa
18	Donata
	Broadsword Hi Pro early cut buffer between experimental plot and existing ryegrass margin
	235m approx.

Figure 5.1. The layout of the 18 replicates of the experimental Donata/Festulolium ley

On 29th September 2020, the fence was removed from the centre of the plots. From 7am on the 30th September 2020, a pit was dug into each replicate using a JCB 3CX Eco backhoe loader. Each pit measured 1 m deep and approximately 1.4 m long and 1 m wide. The cuts into the clay were clean and vertical, and the pits did not crumble. The pits were dug across the fence line, allowing for approximately half a metre in both the grazed and ~1 year ungrazed sections of the cultivar strips. The soil was found to have a clear organic layer in the top 5-10 cm and a clear A-horizon delineation, as seen in Fig 5.2 below. It was observed that below the A-horizon, a layer of agricultural and/or construction material had been deposited, dated to possibly around 50 years before, containing some stones and rubble. It was crushed into a subsurface layer and extremely compacted.

Figure 5.2 Soil horizons clearly visible in the Festulolium/Donata pits

The clay was very compacted from a mix of grazing and machinery, and the waste material was evident. This made sampling difficult with the syringe corers, which had to be used with the aid of a mallet. Apart from the use of a mallet, the syringe corers were used as described in Chapter 2. There corer was driven horizontally into the pit walls at 10, 30, 60, and 90 cm depths, the core ejected into a bag marked with the plot number, volume, depth and pit face (four samples were taken from the north pit face and four from the south), similar to the WG experiments.

Data on root density was also taken by Dr Jennifer Bussell of the Game and Wildlife Conservation Trust at the same time from the same pits. This data has kindly been shared to allow me to compare soil carbon with root density.

Four pits were dug slightly shallower than the rest merely by accident of the machine operator, who left site, which rendered four replicates (plots 1, 3, 4 and 6, or a replicate each of Broadsword, Donata, Perseus and Lofa) without a 90cm depth sample.

5.3.2 Laboratory methods

The laboratory processing for water %, pH, bulk density, and pyrolysis for organic carbon in three fractions were conducted as described in Chapter 2. C:N analysis was omitted at this time for cost and time concerns, with pyrolysis used for the carbon content.

5.3.3 Statistical analysis

Single factor ANOVA was used to compare the pyrolysis fractions of lability to species and depth.

Histograms were created to check all dependent variables had normal distribution.

Linear modelling in R was used to model species and depth as the most important predictor variables, but also BD, water and pH, against the response variables of carbon content by pyrolysis.

Stepwise selection was used to remove variables having no effect on the data.

Models were cross-validated using a training dataset, all variables included.

Generalised linear modelling (GLM) was used to test the predictor variables against the response variables (LOI by pyrolysis at all fractions). The six species were dummy coded and R created the contrast matrix which visualised all the variables, including species. ANOVA was then conducted on the results. Root data from Dr Jenifer Bussell of GWCT was run through another GLM against LOI values, with stepwise selection and a training dataset.

Modelling a combination of mine and Dr Bussell's root data was not an exact depth match. Dr Bussell had slightly different depth sample points and therefore they were matched to the nearest closest depths in my dataset: roots at 15 cm depth points were added to my 10 cm dataset; roots at 40 cm were added to my 30 cm dataset; roots at 70 cm were added to my 60 cm dataset. Our BD data was very similar, but I used data that I had collected from the 10, 30 and 60 cm sample points. Dr Bussell covered grazed and ungrazed areas whereas my samples were confined to ungrazed areas and therefore I limited datapoints to matching ungrazed replicates. Dr Bussell also took samples from the west faces of the pits and I took them from the north and south. I assigned my north samples to Dr Bussell's west samples, deciding that this was likely a variable of no impact. Rather than adding to the existing model, I built a new one where I matched aligning variables from the two datasets. This has a reduced sample size due to fewer replicates, eliminating grazed areas, and one less depth interval (90 cm samples were not taken for roots by Dr Bussell).

5.4 Results

<u>ANOVAs</u>

Several single factor ANOVA tests were run with the main hypothesised drivers (species and depth) against each LOI interval.

At 20-250°C (burning off the most labile fraction), there was a highly significant difference between the amount of organic C lost from the soil of different grass species (p = <0.001). Fojtan had the least weight loss (mean LOI of 11.87%) and Aberniche the highest (mean LOI of 15.76%).

Removing rows with missing 90 cm depth values produces a result of no significant difference between weight loss at different depths (p = 0.29). Removing the whole 90cm sample set also shows no difference in LOI according to 10, 30 or 60 cm sample depth.

At 250-325°C (the somewhat recalcitrant organic C), the C content between the soils of different grass species is highly significant (p = <0.001), with Perseus losing the most weight (3.85%) and Fojtan, again, the least (1.06%).

Removing rows with missing 90 cm depth values shows no significant difference between weight loss and depth (*p* = 0.1). Removing the whole 90 cm set also does not produce any statistically significant difference (*p* = 0.22).

For the most recalcitrant carbon fraction burned off at 325-550°C, there was no difference between species (*p* = 0.59). Removing rows with missing 90cm values and also removing all 90cm values from the dataset also gave no difference in carbon recalcitrance at depth (*p* = 0.17 and *p* = 0.09 respectively).

ANOVA on the total LOI produced a *p* value of <0.001 in differences in organic carbon content between the soil of different grasses. Fojtan had the lowest overall LOI and Aberniche the highest. In decreasing order of loss were Aberniche, Lofa, Broadsword, Donata, Perseus and Fojtan. LOI was again not significantly different between different depths, whether missing values were removed (*p* = 0.31) or the whole 90 cm dataset (*p* = 0.26).

Linear Models (LMs) and Generalised Linear Models (GLMs)

Initially, LMs were modelled without grass cultivar. Depth, BD, water % and pH were predictor variables, and the response variables were the carbon concentrations by way of the LOI intervals.

A LM of LOI at 250°C showed no significant coefficients, the F value was not significant at 0.99, and *p* = 0.42. The R^2 value (how much of the data was explained by the predictor variables) was just 3.1%.

LM for the LOI at the 250-325°C interval shows that soil sample depth is a significant driver of LOI, *p* = 0.008. The *F* value is not significant at 1.92 on 4 and 25 DF, and the total *p* value is 0.11, so depth is the only contributing factor to LOI in this range. The R^2 value shows that 5.2% the data is explained by the predictor variables.

LM for the LOI in the 325-550° interval shows that depth again is significant, *p* = 0.01. However, the *F* value is not significant and nor is *p* (0.15). The R^2 value shows 5.2% data was explained by the predictor variables.

Finally, the total LOI LM showed no significant coefficients, insignificant *p* and *F* values (0.40 and 1.02 respectively), and just 3.2% data explained by the predictor variables.

Using stepwise variable selection in R, running stepAIC (Akaike Information Criterion) showed which variables were affecting the value, and remove those that are having no effect.

- At 250°, only depth was left as a predictor variable, which explains 1.8% the data alone.
- At the 250-325° interval, only depth was left in the model, explaining 5.5% the data.
- At 325-550° interval, only depth was left, explaining 4.5% the data
- For the total LOI, only depth was left, explaining 1.7% the data

Models were cross-validated using a training dataset, but included all the variables again.

- At 250, the explained data increased the R^2 value to 5.8%
- At 250-325, the R^2 value increased to 10.7%
- At 325-550, the R^2 value increased to 12.2%
- And the total LOI, the R^2 value increased to 8.0%

Running GLM (a linear model specifying gaussian link), showed the same values. Only depth has any bearing on any pool of organic carbon content. The pH, water % and BD had no influence on any LOI values.

Grass cultivar, a categorical variable, was dummy coded, and R created the contrast matrix which visualised all the variables including species. ANOVA was conducted on the results of the matrix.

At 250°C, R^2 = 24% and p = <0.001. The ANOVA on the LM gave cultivar as a significant driver of organic carbon, p = <0.001.

At 250-325°C, as well as depth showing as a significant driver of difference in LOI, Fojtan was shown extremely significantly different to other cultivars (p = <0.001). The R^2 value reached 24.7% explained and $p = 5.92 \times 10^{-5}$. The ANOVA on this shows cultivar as highly significant (p = <0.001).

For the most recalcitrant fraction, cultivar once more dropped out of significance. R^2 = 6.6% and p = 0.49. ANOVA on the LM showed depth was still the only significant variable.

For total LOI, the LM showed Aberniche as significant to <0.01; Broadsword significant to 0.12; Donata significant to 0.01; Fojtan highly significant, to <0.001; and Perseus significant to <0.001. The R^2 value is up to 33%. The ANOVA showed a highly significant cultivar effect on LOI, at p = <0.001.

Running the LMs again with a training set altered the R2 values:

- 20-250°C, R^2 = 25.4% (slight increase)
- 250-325°, R^2 = 22.2% (slight decrease)
- 325-550°, R^2 = 8.4% (slight increase)
- And for total LOI, R^2 = 34.24% (slight increase).

Throughout the discussion, multiple R^2 values are used. Table 5.2 below compares the multiple R^2 to adjusted R^2 values from each model. Training the dataset doesn't produce two R^2 values or a p value, only RMSE, R^2 or MAE values.

Table 5.2 Comparisons of R^2 values across GLMs

Model	**With training**	**Multiple R^2**	**Adjusted R^2**
LOI up to 250, no species or root data	No	3.1%	-0.0004%
LOI up to 250, no species or root data	Yes	5.8%	-
LOI up to 250 including root data and species	No	22.8%	2.6%
LOI up to 250 including root data and species	Yes	30.8%	-
LOI 250-325, no species or root data	No	5.8%	2.6%
LOI 250-325, no species or root data	Yes	10.7%	-
LOI 250-325 including root data and species	No	30.5%	12.3%
LOI 250-325 including root data and species	Yes	37.1%	-
LOI 325-550, no species or root data	No	5.2%	2.2%

LOI 325-550, no species or root data	Yes	12.2%	-
LOI 325-550 including root data and species	No	9.3%	-14.3%
LOI 325-550 including root data and species	Yes	28.5%	-
Total LOI, no species or root data	No	3.2%	0.0008%
Total LOI, no species or root data	Yes	8.0%	-
Total LOI including root data and species	No	29.1%	10.5%
Total LOI including root data and species	Yes	24.8%	-

Root GLMs

New GLMs including root data were performed on a smaller dataset given the mismatch in the depth and grazing aspects of the data.

GLM with labile C as the response variable produced Donata, Fojtan and Perseus as significant drivers ($p = 0.001$, $p = 0.023$ and $p= 0.029$ respectively) but cultivar is already understood as a driver. The new variables are soil porosity and mg root kg soil^{-1}, neither of which are significant drivers of labile C. The ANOVA table of the GLM produced no significant drivers or interactions.

GLM with mid-lability C as the response variable produced only Fojtan as a significant driver (p = 0.014) which is known, but soil porosity or root biomass are not significant predictors. ANOVA on the results of the GLM show cultivar as driver and no other variables.

GLM with recalcitrant C showed no significant drivers, including soil porosity and root biomass. ANOVA on the GLM results also showed no drivers.

Fojtan and Perseus showed as significant drivers of total LOI, but not root biomass or soil porosity, and only cultivar was identified in the ANOVA.

5.5 Discussion

5.5.1 Labile C ANOVA

At 20-250°C, the temperature interval which ignites the most labile C fraction, single-factor ANOVA showed a highly significant difference between the amount of organic C lost from the soil of different grass species ($p = <0.001$). Fojtan had the least weight loss (mean LOI of 11.87%) and Aberniche the highest (mean LOI of 15.76%) (Fig. 5.3.)

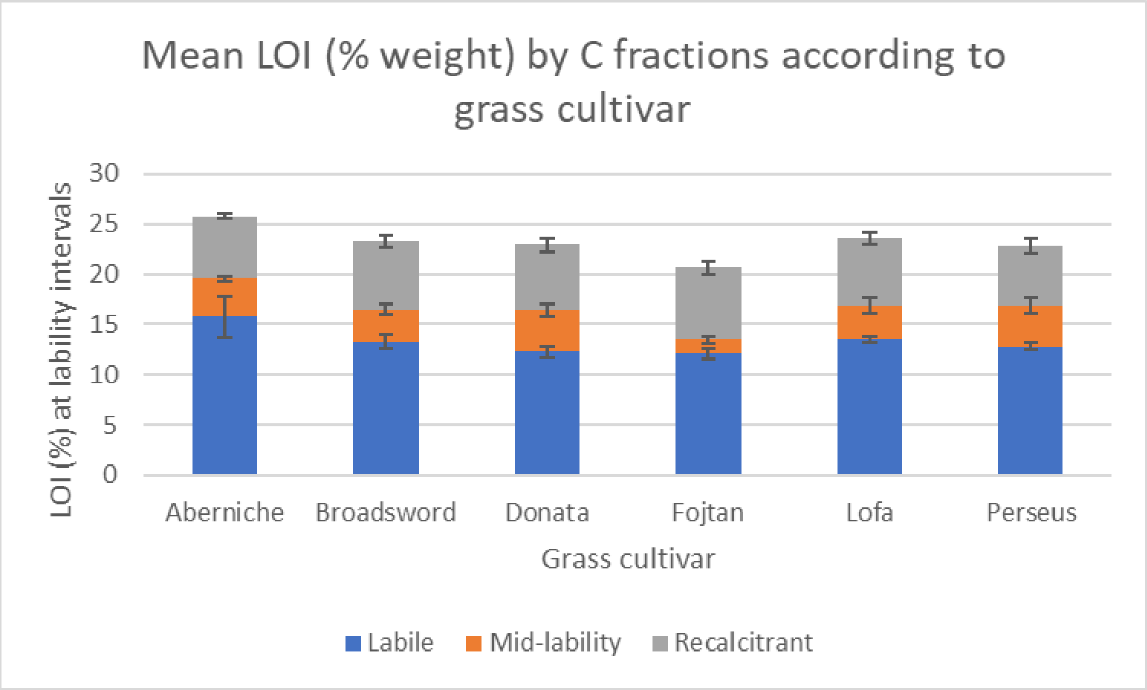


Figure 5.3 Mean LOI (%) by C fractions according to species

The losses of labile C by individual species are significantly different (Fig. 5.3 with SE) and this is driving a species effect in the ANOVA. Species are likely to be driving differences in soil C by the action of roots, which perturb the soil, create pores for water and air, release exudates, and die off seasonally which provides fresh subsoil OM (Dodd *et al.*, 2011; Kell, 2011; Mellado-Vázquez *et al.*, 2016; Pausch and Kuzyakov, 2018). Roots also lead to differences in other soil parameters and could be influencing labile C indirectly by mediating water supply to microbial actors (Erktan, Or and Scheu, 2020); by decreasing soil BD and thereby improving nutrient transport (López-Fando, Dorado and Pardo, 2007); or by absorbing nutrients which might otherwise cause a decrease in pH which is linked to an increase in C mineralisation (Chen *et al.*, 2018).

When rows with missing 90 cm values were removed from the analysis, species remained very highly significant, p = <0.001. Perseus, rather than Aberniche, lost the most weight when 90 cm values were removed, which indicates that a higher proportion of the total C is stored in the labile fraction at 90 cm depths in Aberniche. This is evidenced by the mean losses in Table 5.3, where Aberniche soil holds a mean 61.07% of its total C stock in the labile fraction, compared to 55.95% under Perseus. Species differences in C lability at depth are also likely driven by root biomass (Bernal *et al.*, 2016), but also by microbial action (de Oliveira, Oliveira and Xavier, 2016) and nutrient leaching (Ding *et al.*, 2016).

Depth interval showed no significant influence on weight loss at any depth, whether rows with missing 90 cm values or the whole 90 cm portion of the dataset were removed for analysis, (p = 0.29). While ANOVA is not detecting a difference between C lability and depth, there is a small variation (see Fig. 5.4 below). With samples taken over a longer period of time, this depth difference might become statistically observable (Poeplau *et al.*, 2011) It also ties into results discussed in Chapter 4 of this thesis, where depth did not have a statistically significant effect on the lability of the C in the soil, but patterns were evident in graphical representation and through mediating factors including root biomass. The results of root biomass are discussed further below.

5.5.2 Mid-lability C ANOVA

At 250-325°C (mid-lability organic C), the C content between the soils of different grass cultivars is highly significant (*p* = <0.001), with Perseus losing the most weight (3.85%) and Fojtan, again, the least (1.06%). The comparative loss on ignition at mid-lability is illustrated in Fig. 5.3, where some overlap between the SE in mid-lability C is observed in some cultivars, e.g. Perseus overlaps with Lofa and Donata. Despite some overlaps, there is enough difference detected to drive high significance in the ANOVA, indicating differences in the way each cultivar stores mid-lability C. This will be discussed further with regards to potential impacts from root biomass and depth below. As a mean proportion of the total C stored in the mid-lability fraction, Donata holds the most (18.10% total LOI) with Perseus close (17.83% total LOI), and Fojtan by far the least (6.81%) (Table 5.3). This suggests that Perseus and Donata are more effective than other cultivars for contributing a greater proportion of biomass-derived C to the mid-lability pool.

Removing rows with missing 90 cm depth values shows no significant difference between mid-lability LOI and depth (*p* = 0.1). Removing the whole 90 cm set also does not produce any statistically significant difference (*p* = 0.22). This means again that depth is not acting as a significant driver on the fractions of carbon stored. Like WG1 and WG2, the Leicestershire experiment is not providing evidence for a relationship between depth and carbon recalcitrance. Slight differences do not show as significant, and this may be due to mid-lability compounds taking a longer time to develop, potentially longer that the individual cultivar replicates have been established. A further explanation for this may be the smaller temperature interval for mid-lability C burning, which means potentially the pool of data is smaller which reduces the ability of ANOVA to detect differences and reduces the precision of the dataset.

5.5.3 Recalcitrant C ANOVA

ANOVA shows that species are not driving a difference in recalcitrant C (*p* = 0.59). From Fig. 5.3, it is evident that Fojtan has a higher LOI in the recalcitrant fraction but all other species were approximately equal in their mid-lability losses. Table 5.3 offers both mean loss on ignition for each species, with SE, and the mean proportion of the total weight loss which burned off in each pool of recalcitrance. Potentially, the difficulty in detecting differences in recalcitrant C when labile and mid-lability C show strong significance is a result of the time and OM inputs of the ley. This Leicestershire ley had been in place for four years and was at the end of its life before being converted to a herbal ley experiment with higher diversity. One timepoint after four years does not give a comparison to a baseline (here, Broadsword was the control, but no soil data pre-planting was available) or any clarity on seasonal and longer-term changes in total or fractional C. However, labile C compounds are produced on shorter timescales and from 'young' organic matter inputs (Kramer and Gleixner, 2008) and the formation of recalcitrant compounds can take place on a scale of decades, and therefore be harder to detect (Lehmann *et al.*, 2020)

Table 5.3 shows that, proportional to total weight loss, Fojtan soil contains the most recalcitrant C at a mean 34.53% of total C. Aberniche contains the least, with recalcitrant C 24.10% the total C. Despite this, patterns are not consistent enough for ANOVA to detect, although that patterns are showing is a positive step towards being able to recommend cultivars for soil health in grass leys.

Table 5.3 Species division of mean weight lost on ignition for each C fraction (%) and mean proportion of the lost weight devoted to each C fraction

Grass cultivar	**% weight LOI / proportion of weight lost by fraction**	**Labile C LOI**	**Mid-lability C by LOI**	**Recalcitrant C by LOI**
Aberniche	*% sample weight lost*	15.75±2.01	3.82±0.20	6.21±0.25
	% of the total loss in each fraction	61.07±2.55	14.83±1.23	24.10±1.61
Broadsword	*% total weight lost*	13.30±0.68	3.15±0.51	6.83±0.58
	% loss according to fraction	57.13±2.71	13.51±2.41	29.36±2.23
Donata	*% total weight lost*	12.24±0.53	4.15±0.59	6.54±0.67
	% loss according to fraction	53.39±1.35	18.10±2.50	28.51±3.01
Fojtan	*% total weight lost*	12.10±0.51	1.40±0.40	7.12±0.70
	% loss according to fraction	58.66±2.27	6.81±1.70	34.53±3.00
Lofa	*% total weight lost*	13.53±0.24	3.37±0.79	6.70±0.64
	% loss according to fraction	57.34±0.63	14.28±3.21	28.39±3.07
Perseus	*% total weight lost*	12.78±0.38	4.07±0.76	5.99±0.78
	% loss according to fraction	55.95±0.90	17.83±3.26	26.22±3.61

Removing rows with missing 90 cm values, or removing all 90 cm values from the dataset, also produced no difference in carbon recalcitrance at depth ($p = 0.17$ and $p = 0.09$ respectively). Depth, as in WG1 and WG2, is not showing as a significant driver of recalcitrant C. However, WG1 provided some evidence for linking shallowness to C lability. This has not been replicated in the Leicestershire experiments. However, there is no time progression in this experiment which means that seasonal differences will be lost, and as most of the processes governing the mineralisation of subsoil C take seasons or years (Barré *et al.*, 2010; Das *et al.*, 2019; Biffi *et al.*, 2022), this is an important limitation of this experiment.

5.5.4 Total LOI ANOVA

ANOVA on the total LOI produced $p = 1.62\text{x}10^{-6}$ in cultivar differences in total LOI (a proxy for total organic carbon content). Fojtan had the lowest overall LOI and Aberniche the highest (Fig. 5.3). In decreasing order of loss were Aberniche, Lofa, Broadsword, Donata, Perseus and Fojtan. LOI was again not significantly different between different depths, whether rows with missing values were removed ($p = 0.31$) or the whole 90 cm dataset ($p = 0.26$).

Fig. 5.4 below illustrates the differences between depths across species in the labile, mid-lability, recalcitrant and total LOI. Aberniche stores the most C in the labile fraction and the most overall, as it has the highest weight loss (%) at both 250°C and 550°C, and Fojtan the least C overall when depth is not taken into account (Fig. 5.3). However, accounting for depth in Fig. 5.4 shows there are fluctuations in how much total and pooled C is stored throughout the depth profile. There is relative consistency across the soil supporting all other cultivars and across all depths. Depth did not show as significant with regards to any fraction of C in the ANOVAs, however, a pattern is still observable in Fig. 5.4. Broadsword, Donata, Lofa and Perseus see consistent, if small, decreases in total C with depth. However, Aberniche has an increase of the proportion of C which is in the labile pool at 30 cm, compared to the labile proportion at 10 cm. Although not significantly different, this is useful supporting evidence for the theory that lability decreases with depth, and either a larger sampling pool or a longer period of time over which to sample may have clarified whether this is a seasonal or long-term change, and whether Aberniche is more effective at sequestering C at lower depths than other grass cultivars. Fojtan also stores a lower proportion of labile and mid-lability C at 30 cm than at 10 cm, but the proportion of total C stored in a recalcitrant fraction is higher at 30 cm than 10 cm and higher at 60 cm than 30 cm. Fojtan provides the first evidence linking depth to C recalcitrancy found across WG1, WG2 and Leicestershire experiments discussed in Chapters 3, 4 and 5 of this thesis. Previously, only support for shallowness and lability has been evidenced and not the reverse. Although not a significant difference, the identification of this pattern is still important information. It matches with evidence in the literature of a relationship between depth and recalcitrance (Rovira and Vallejo, 2002; Lorenz and Lal, 2005).

Aberniche and Fojtan are the only species which store more C overall at 60 cm than 10 cm. This is a positive indication that some grass cultivars are more active in storing C at depths and that these cultivars could play a part in carbon sequestration in existing grasslands, and positive impacts on the proportion of C stored in deeper soils could be supported by adding seeds into grassland ley mixes.

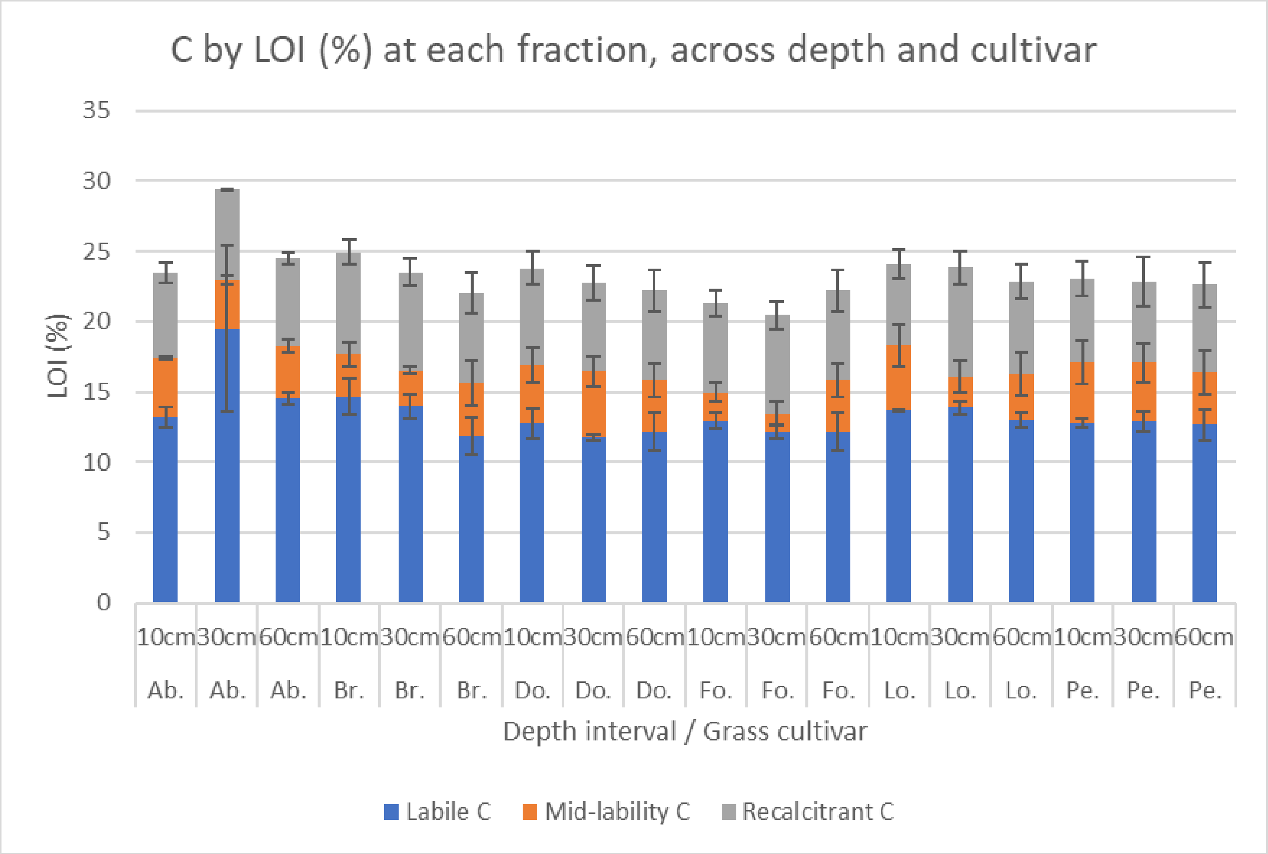


Figure 5.4. Depth and cultivar differences in fractions of C by LOI (%)

5.5.5 Labile C GLMs

Without species, a linear model produced no significant coefficients, the F value was not significant at 0.99, and $p = 0.42$. The R^2 value (how much of the data was explained by the predictor variables) was just 3.1%. Using stepwise variable selection, stepAIC (Akaike Information Criterion) showed that only depth was a predictor variable, removing all others. After cross validation, depth explained 5.8% the data (the R^2 value). GLM produces the same values with only depth as a predictor of labile C.

For GLM including the cultivar category, R^2 increased to 25.4% after cross validation, and $p = 8.9\text{x}10^{-5}$. The ANOVA on the LM gave grass cultivar as a significant driver of organic carbon, $p = 1.99\text{x}10^{-5}$. It can be inferred from the R^2 values of both models that cultivar drives about 14% the change in labile C and potentially more, given that the number of variables has also increased and therefore the proportion shared by each has also changed. Combining this result with the ANOVA on cultivar means that the majority of these differences are likely to be driven by Aberniche and Perseus cultivars.

It is expected that some or all of the other predictor variables (pH, BD, water %) are not as influential as depth or cultivar, as species differences and a depth profile for C are established well in the literature even in recently converted treatments (Rasse *et al.*, 2006; Skinner *et al.*, 2006; Guan *et al.*, 2016). In this experiment, samples are all taken from the same field which has undergone decades of homogenous treatment, and has a clayey soil difficult to permeate. Processes may be taking place on timescales relevant to temporary leys, but the effect of these may be mediated by the legacy effect of previous monocultures. While processes which change parameters including pH and BD can be species-driven, all of the species tested here are Festulolium crosses, cultivars or cross-breeds of *Lolium perenne*. It is not unexpected that root exudates, rates of rhizodeposition or associated microbial communities influencing basic soil parameters are not significantly different between closely related species, although some might be. This is because differences in root biomass is the main subsoil difference between cultivars, where the crossbreeds are bred mainly for increasing drought tolerance. Root biomass has been suggested as a key driver of subsoil C change even on short timescales (Skinner *et al.*, 2006; Steinbeiss *et al.*, 2008). This is alongside factors such as field compaction from years of cultivation and a legacy effect from monocultures and artificial inputs. The analysis to test whether species, and differences in root biomass, can influence total C and C fractions are also in spite of the legacy of monoculture and inputs in the field. However, this experiment duration and its establishment on previously arable soil, is representative of many leys which are subject to practices which increase the rate of soil health decline, it is important to understand whether C change can be driven on homogenised and damaged soils over periods of <5 years.

Figure 5.3 above illustrates the broad pattern, without depth as a factor, of the relationship between cultivar and labile C. Figure 5.4 illustrates variation with depth. These are the two drivers picked up in the GLMs and the most important factors to consider with regards to a ley planting regime of a maximum 95% grass (5% leys must be legume species), with improving labile soil C as a goal. Increased labile C has been linked to improved crop growth when the ley is put back into rotation (De Moraes Sá *et al.*, 2014), but the longevity of the legacy effect is not well established (Zhao *et al.*, 2020). It is likely to be influenced by many factors including artificial and organic inputs, soil texture and structure, livestock units and grazing type, and crop type in rotation (Liebig *et al.*, 2010; Mcsherry and Ritchie, 2013; Bucka *et al.*, 2019).

5.5.6 Mid-lability C GLMs

No basic parameters were found to be good predictors of mid-lability C in any of the GLMs. As discussed in section 5.5.5, basic parameters are not necessarily expected to change rapidly, especially in relation to mid-lability C, which is a smaller interval of recalcitrance than labile or recalcitrant C. Therefore it may be harder to detect patterns as sample size is more restricted. The more important predictors of C are depth and cultivar, as changes to cultivar(s) in grass leys is the treatment being investigated for its potential to affect C and the expectation is that this will mainly be controlled via root biomass by physical (Cui *et al.*, 2019) or chemical (De Neergaard and Gorissen, 2004; Fornara and Tilman, 2008) influences.

Without species, the LM for the LOI at the 250-325°C interval shows that the depth at which soil is collected is a significant driver of LOI, $p = 0.008$. The F value is not significant at 1.92 on 4 and 25 DF, and the total p value is 0.11, so depth is the only contributing factor to LOI in this range. The R^2 value shows that 5.2% the data is explained by the predictor variables. StepAIC removed all variables except depth. After cross validation, depth explained 10.7% the data. GLM produces the same values with only depth as a predictor of mid-lability C.

For GLM including cultivars, Fojtan was added to depth as a significant driver, with $p = <0.001$. The R^2 value reached 22.2% after cross validation, and $p = 5.92 \times 10^{-5}$. The ANOVA on this shows cultivar as highly significant ($p = 5.14 \times 10^{-5}$). By comparing the two models, it can be inferred that cultivar is responsible for driving around 11% the change in mid-lability C. Linking the GLM results to those of the mid-lability ANOVA, where difference was also driven by Perseus, which lost the most weight.

Figure 5.3 above illustrates the broad pattern, without depth as a factor, of the relationship between cultivar and mid-lability C. As Fojtan showed in the GLM as the most significant driver, Fig. 5.3 shows that this is because soil supporting Fojtan holds significantly less of its total C in the mid-lability fraction, not more, while the other cultivars are fairly consistent. This is shown in Table 5.3 where the mean proportion of Fojtan's total C stored in the mid-lability fraction is 6.81%, compared to 13-18% in other cultivars. Perseus, identified as a driver in the ANOVA, has a mean proportion of total weight loss in the mid-lability fraction of 17.83%, which is a close second-highest. It is likely that an interaction, even if statistically weak, with another variable brings Perseus into significance. This could be related to depth or root biomass, or microbial action, as discussed in later sections.

Figure 5.4 illustrates variation with depth. ANOVA suggested no significant relationship between depth and mid-lability C, but the increased statistical power of GLM picks up depth as a significant predictor of mid-lability C. Depth and cultivar work together at mid-lability scale in Fojtan, where Fojtan is the only cultivar in which we see the pattern of not only increasing total C at every depth interval, but an increasing proportion of that stored in the mid-lability fraction. Perseus, by contrast, shows almost no difference in total C or mid-lability fraction across either 10 cm, 30 cm or 60 c depth interval. Figure 5.4 does, however, visualise a difference in the LOI in the mid-lability fraction over the depth profile in Aberniche, which has less at 60 cm than shallower samples; in Broadsword, which also has slightly less at 60 cm than 10 or 30 cm; and in Lofa, which has slightly more at 60 cm than at 30 cm, but 10 cm remains the largest LOI for mid-lability. Crucially, these have overlapping SE which mean the pattern can be visualised but is not strong enough to show a significant difference.

Cultivar and depth are the two drivers picked up in the GLMs and the most important factors to consider with regards to a ley planting regime of a maximum 95% grass (5% leys must be legume species), if improving labile soil C is a goal. Increased labile C has been linked to improved crop growth when the ley is put back into rotation (De Moraes Sá *et al.*, 2014), but the longevity of the legacy effect is not well established (Zhao *et al.*, 2020). It is likely to be influenced by many factors including artificial and organic inputs, soil texture and structure, livestock units and grazing type, and crop type in rotation (Liebig *et al.*, 2010; Mcsherry and Ritchie, 2013; Bucka *et al.*, 2019).

5.5.7 Recalcitrant C GLMs

Without cultivars as a predictor, the LM for LOI in the 325-550^o interval shows depth is significant, *p* = 0.01. However, the *F* value is not significant and nor is *p* (0.15). The R^2 value shows 5.2% data was explained by the predictor variables. StepAIC removed all variables except depth. Cross validation increased the R^2 value to 12.2%. GLM produces the same values with only depth as a predictor of recalcitrant C.

Adding cultivars to a new GLM did not produce a significant result, and consequently the total proportion of data explained by the variables fell, because the number of variables had been increased with little to no extra value added in statistical significance. Depth remained the only good predictor of recalcitrant C. After cross validation, the R^2 value was 8.4%. ANOVA on the cultivar-inclusive GLM showed depth remained the only significant variable. Despite depth remaining in the stepwise models, the R2 values for the proportion of data explained by the variables is low. This concurs with the initial ANOVA that showed species is not driving a difference. This is despite Fojtan soil storing a higher proportion of C in the most recalcitrant fraction (34.53% total C is in the recalcitrant fraction compared to 24-29% for the other cultivars) (Table 5.3). Patterns are not consistent enough for ANOVA to detect, although that some weak patterns are showing is a positive step towards being able to recommend cultivars for soil increasing the ratio of recalcitrant to labile C in grass leys. The patterns are weak: Fig. 5.4 shows that, accounting for SE, there are many overlaps between the recalcitrant fraction of C from almost all the cultivars. The majority of difference is driven by labile C and at first glance, Fig. 5.4 shows little difference between any depth and cultivar in recalcitrant C. This is likely to be because any processes which drive differences in recalcitrant C, if it is possible between closely-related variants, are likely to take place over much longer timescales than the ley was established (Kramer and Gleixner, 2008; Lehmann *et al.*, 2020). Additionally, a limitation of this experiment was a lack of any time progression element, so if any gains have been made in recalcitrant C over the last four years, they are not being detected in the models from one timepoint.

Depth showing as a significant predictor of recalcitrant C in the GLM is reflected in Fig. 5.4 above, where depth differences are more easily discernible and significant than cultivar differences. Table 5.3 gives further detail. Contradicting the ANOVA result (*p* = 0.09 for the 0-60 cm dataset), the GLM has enough statistical power to detect slight differences in recalcitrancy relating to depth. This is the second piece of evidence from this Leicestershire experiment which does link recalcitrant C to depth, as discussed above in section 5.5.4. No evidence was found in WG1 and WG2 to link recalcitrance to depth, which indicates the potential for four years being enough to link recalcitrance with depth where two years is not. WG1 did establish a link between shallowness and C lability, but not depth and recalcitrance. Taking these results together, there is evidence for a recalcitrance profile in the soil. This is important information because if this can be enacted by some ley species or cultivars over four years, it may be applicable to storing soil C in less labile forms which reduces the loss of soil C to the wider environment.

A lack of time points in this experiment means seasonal differences go undetected, despite a seasonal influence on soil C (Skiba *et al.*, 2013), which brings greater detail to the map of C fluctuation over time. The only time comparison is with WG1 and WG1, which is not a fair one with equivalent conditions. Therefore no assumptions can be made on the timescale of how depth comes to develop a relationship with recalcitrant carbon. As most of the processes governing the mineralisation of subsoil C take years (Barré *et al.*, 2010; Das *et al.*, 2019; Biffi *et al.*, 2022), a lack of time clarity is a limitation of this experiment. However, the evidence provided for a depth-recalcitrance relationship may still be useful in creating a positive management plan for a greater proportion of recalcitrant carbon in soil.

5.5.8 Total LOI GLMs

Finally, the total LOI LM showed no significant coefficients, insignificant *p* and *F* values (0.40 and 1.02 respectively), and just 3.2% data explained by the predictor variables. StepAIC removed all variables except depth, and after cross validation, 8.0% the data was explained by these variables. GLM produces the same values, with only depth as a predictor of total C by LOI.

For total LOI including cultivars, the GLM showed Aberniche as significant to <0.01; Broadsword significant to 0.12; Donata significant to 0.01; Fojtan highly significant, to $1.09\text{x}10^{-10}$; and Perseus significant to <0.001. Only Lofa was excluded. The R^2 value is up to 33%. The ANOVA showed a highly significant cultivar effect on LOI, at $p = 4.29\text{x}10^{-8}$. Therefore, we can infer that cultivar has a powerful predicting effect on total LOI, with the model explaining up to 34.2% the data after cross-validation. Cultivar may predict around 26% the data in the model. This confirms the previous ANOVA result.

It is important that depth is a predictor of total LOI as well as fractions, because it suggests a pattern (even if not significant at a single timepoint) in C transitions between fractions with depth. Fig. 5.4 shows the fluctuations in total C across depths, with a particular fluctuation seen in the high carbon content at 30 cm under Aberniche compared to 10 and 60 cm samples. Other cultivars also show a depth pattern, with Aberniche and Fojtan the only cultivars with an increase in C with depth (Fojtan has a slight drop between 10-30 cm but an increase between 30-60 and overall increase between 10-60 cm depths). All other cultivars have the highest total LOI at 10 cm, which confirms that more C is stored in shallower horizons than deep horizons, and supports the theory of a carbon depth profile (Jobbágy and Jackson, 2000).

Depth is related to recalcitrance because horizons more vulnerable to outside influences such as plant roots, mechanical intervention, erosion, precipitation and grazing or poaching by animals are expected to have a higher turnover of C as these are disruptive to processes which might form recalcitrant C, but influence the abundance or richness of OM inputs. Labile C, which has been established as having a relationship with shallowness in WG1 and Leicestershire, is a result of ‘young’ inputs (Kramer and Gleixner, 2008; Phillips *et al.*, 2012), which are more heavily contributed to by surface-level biomass by the nature of plant growth and resource partitioning.

Cultivar as a strong predictor of total LOI supports the hypothesis that different dominant surface plants maintain differences in belowground interactions. On short timescales, these are likely to be driven mainly by root biomass, length or density (Roberts and Johnson, 2021), and potentially other factors such as exudates supporting a different microbial community (Poirier, Roumet and Munson, 2018). Fig. 5.3 shows differences in overall LOI between cultivars, where Aberniche and Fojtan are significantly different from all others, with no SE overlap. That differences are detected with only one timepoint in the GLM shows potential in recommending cultivars to increase total C in grass leys. Without a baseline, however, we cannot establish whether the four years that this ley was established is enough to achieve a total C improvement from the soil C concentration before the ley was planted. We also cannot rule out seasonal fluctuations as potential drivers of change, which would fluctuate over time and do not accurately describe overall increase/decrease in total LOI.

Therefore, limited sampling time limits the recommendations for management which can be made from this experiment and further data is required to feed into any policy modifications around the length of time a ley should be established for, or the cultivars recommended for soil C manipulation.

5.5.9 Root GLMs

Labile C and root biomass

GLM with labile C as the response variable produced Donata, Fojtan and Perseus as significant drivers (*p* = 0.001, *p* = 0.023 and *p* = 0.029 respectively), but cultivar is already understood as a driver and has been discussed above. The new variables of interest are soil porosity and mg root kg soil^{-1}, neither of which are significant drivers of labile C. The ANOVA table of the GLM produced no significant drivers or interactions. The lack of a distinct relationship between labile C and root biomass is illustrated below in Fig. 5.6, which compares total root biomass (mg kg soil^{-1}) to C by fraction.

Lofa has the highest root biomass in the 10 cm depth interval, with a mean of 296.46±135.99 mg kg soil^{-1}, although not the highest mean weight loss at 10 cm depth (which is Broadsword at 14.66±1.30%). This means high root biomass is not matching to high LOI at 10 cm in the labile fraction, providing no evidence for a link between shallow depth and lability. This link was evidenced in Chapters 3 and 4 of this thesis. There is also no link established in this experiment between higher root biomass and lability. Perseus has the lowest root weight at 10 cm, 26.67±62.23 mg kg^{-1}, although not the least % LOI, again not supporting a link between shallowness and lability or root biomass and lability.

At 30 cm depths, Fojtan is notable for its peak in mg root kg soil^{-1}, low LOI, and the highest root:labile C ratio. This is not surprising, as Fojtan was driving species differences across labilities in the GLMs. It may be that the large SE has ruled out a detectable predicting effect of root biomass. However, there is a link, even if not statistically significant, between root biomass and mid-lability C in the Fojtan cultivar. Aberniche shows the high proportion of LOI at 30 cm but this is not related to a relatively low mg root kg soil^{-1} value.

At 60 cm depths, there is more consistency between cultivars in the proportion of weight lost in labile C, but Perseus shows the lowest ratio of root:labile C and also showed up as a species driver in the GLMs.

This result, along with the results from mid-lability and recalcitrant C GLMs comparing carbon to suboil plant biomass is explored further in below, in section 5.5.10.

Mid-lability C and root biomass

GLM with mid-lability C as the response variable produced only Fojtan as a significant driver (p = 0.014) which is known, but soil porosity or root biomass are not significant predictors. ANOVA on the results of the GLM shows cultivar as a driver (as discussed above), and no other variables. The lack of a relationship between mid-lability C and root biomass is illustrated below in Fig. 5.6. Potential mediators of this effect are discussed below in section 5.5.10.

There is much more variation in 10 cm weight loss between cultivars at mid-lability than in labile C, but Lofa which has highest root weight also has the highest LOI (4.63±1.49% total soil weight lost). This could go some way towards establishing a link between root biomass and % C stored in mid-lability fractions, but according to GLMs, this is not a consistent enough pattern to detect root biomass as a significant predictor of mid-lability C. If there is a link, it is likely that it is mediated through the microbial activity discussed above.

At 30 cm depth, Donata and Perseus show high LOI but also high SE, negating a significant difference between them, with low root biomass. Fojtan is the most notable cultivar at 30 cm for mid-lability C, with the lowest LOI (1.27±0.87) and highest root biomass (254.38±153.97) but this is clearly not a consistent pattern. SEs overlap across most cultivar LOI values and root biomass values. If Fojtan is indicating an efficient root to mid-lability C ratio, this could have positive implications for Fojtan as a C-sequestering grass cultivar. However, as discussed above, biomass itself is not a significant contributor to the C effect and any link between subsoil biomass and mid-lability C is likely to be indirect via microbial processes.

At 60 cm, all LOI values are fairly consistent across grass cultivars, while root biomass fluctuates, indicating no link between biomass and mid-lability C; meanwhile SEs are large and overlapping, providing no support for a pattern of cultivar influencing the proportion of mid-lability C at depth.

Recalcitrant C and root biomass

GLM with recalcitrant C as the response variable showed no significant drivers, including soil porosity and root biomass. ANOVA on the GLM results also showed no predictors. The lack of relationship between recalcitrant C and root biomass is illustrated below in Fig. 5.5. Potential drivers are explored together with labile and mid-lability C in section 5.5.10 below.

Fojtan and Perseus showed as significant drivers of total LOI, but not root biomass or soil porosity, and only cultivar was identified as a significant predictor variable in the ANOVA. Allied to the same overall root biomass figures, the progression of LOI over depth shows that Lofa has the highest root biomass to recalcitrant LOI ratio 10cm, indicating there may be some relationship between high root biomass and a higher proportion of recalcitrant C in shallow soil. However, Lofa does not have the highest recalcitrant LOI overall, which is Broadsword, which has the second-lowest root biomass value. These contrasting facts mean it is difficult to discern any pattern, even an insignificant one undetectable to the GLMs. Recalcitrant C is fairly consistent between cultivars across 30 and 60 cm depths, with slightly higher LOI at 30 cm than 60 cm. This does not support the hypothesis that greater recalcitrant C is stored with increasing depth. Fojtan shows as having high root biomass at 30 cm compared to other cultivars, and a comparable recalcitrant LOI, showing that at 30 cm Fojtan may exercise some influence over a higher proportion of C stored in recalcitrant compounds mediated by root biomass. At 60 cm, there is little discernible pattern but Perseus has comparable LOI values to other cultivars and a lower biomass, which again contradicts the potential link between depth and recalcitrance indicated by Fojtan, and indicates that cultivar has a stronger influence than depth on carbon recalcitrancy.

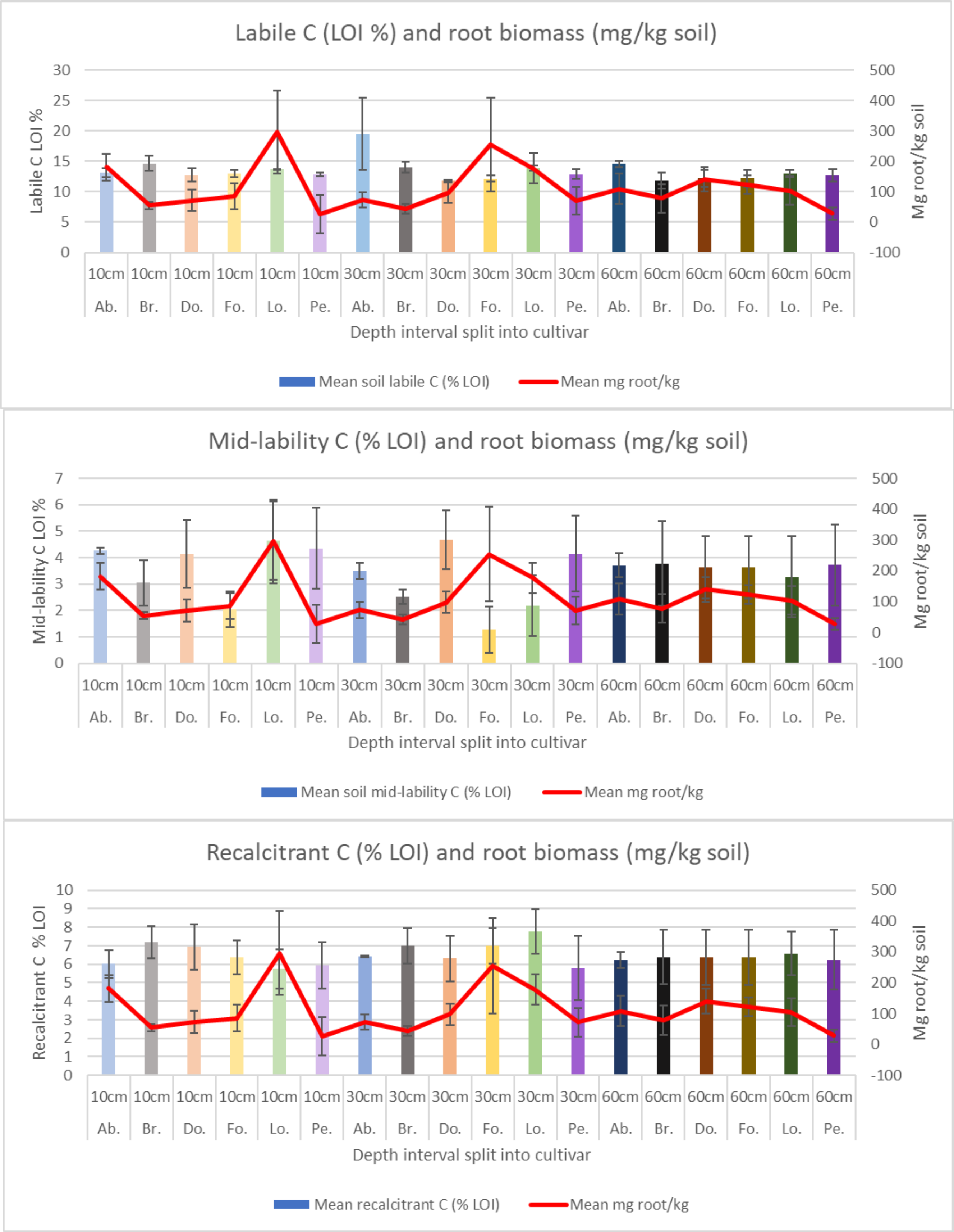


Figure 5.5 Fractions of C (% LOI) and relationship to root biomass (mg kg soil^{-1})

Total C by LOI and root biomass

The lack of patterns in labile, mid-lability and recalcitrant fractions against root biomass are reflected in the lack of relationship shown between total LOI and root biomass. No GLM reflected any significant prediction effect of root biomass on any fraction of C or total C. The lack of correlation is illustrated below in Fig. 5.6, where depth intervals are split by cultivar (darker colour = deeper soil, as in Fig. 5.7, colour-coded by cultivar).

Aberniche lost its greatest proportion of total C at 30 cm depth, but this is accompanied by a large SE overlapping with several other cultivars. 10 cm and 60 cm C content in Aberniche were comparable (23.47±0.12 compared to 24.48±0.62 respectively), providing no evidence of this cultivar sequestering more carbon in the most bioactive O horizon, as might be expected. Additionally, highest root biomass in Aberniche is at 10 cm depth whereas highest LOI is at 30 cm depth, showing no link between total C and root biomass in Aberniche.

Broadsword shows relative consistency in total LOI across all depths, indicating that Broadsword has no particular depth profile with regards to total C and is not one of the cultivar drivers of the ANOVA significant result. Broadsword also did not show in the GLM as having a significant predicting effect on C. Broadsword also has a consistently low mg root kg soil^{-1} value, indicating no correlation between depth, C, or root biomass.

Donata soil had a very low proportion of its total C at 10 cm depth, storing more C at 30 and 60 cm depths. This does indicate a positive outcome for storing C at greater depths in a ley of Donata, especially as LOI SE values are low. The low LOI value at 10 cm accompanies a low root biomass value (unlike Broadsword which has high LOI and low root biomass), making a cultivar difference in LOI clear, and establishing that any observable relationship between root biomass and C is cultivar-specific and not a general rule.

Fojtan has previously been shown as a significant driver, and while it does have high root biomass values in comparison to other cultivars at 30 and 60 cm depths, it does not at 10 cm depths. Fojtan soil stores much more C at deeper depths than at the 10 cm interval, but this is not accompanied by high root biomass at any stage. Fojtan is suggested as a positive grass cultivar for storing C in more recalcitrant fractions, although this is unrelated to root biomass on the limited timepoint of this experiment, and the gain in recalcitrant C is not reflected in an overall increase in C compared to other cultivars.

Lofa stores a comparatively high proportion of C at every depth interval, but a very large SE value at 10 cm, suggesting inconsistence. This why GLM failed to establish a link between Lofa biomass and total C, and why Lofa does not support the hypothesis that shallow depth accompanies more C and a higher proportion of labile C. Lofa root biomass also falls at every depth interval (296.46+135.99 mg kg soil^{-1} at 10 cm; 176.99+49.26 mg at 30 cm; and 104.03+45.76 mg at 60 cm), the only cultivar to do so. Donata shows the opposite result, with root biomass climbing consistently with depth, while all other cultivars are heaviest at 30 cm. Alongside this consistent fall in Lofa biomass is a consistent fall in mean total C, but only slightly and again with overlapping SE values: 24.09±0.46% at 10 cm, 23.84±0.15% at 30 cm, and 22.86±1.30% at 60 cm depth. Lofa may support the hypothesis of roots influencing total C if the experiment had more than one sample point, but currently there is not enough information in the model to draw a link.

Perseus has relatively consistent LOI values compared to other cultivars, and low SE values. However, at 10 cm a high LOI accompanies a very low root biomass weight; at 30 cm, Perseus has its highest root biomass weight with similar LOI values to other depths; and root biomass falls again at

60 cm with no significant fall in soil C accompanying. No pattern between LOI and root biomass is discernible in Perseus, and it does not show a comparative positive effect on LOI over other cultivars.

No pattern is visible across any cultivar with regards to biomass and total C, even broad scale patterns in means which are not significant enough to show in a GLM. In some cases, the SEs were so large that they outstripped the mean value, so it is not surprising that any differences visible in the means were undetectable in the GLMs.

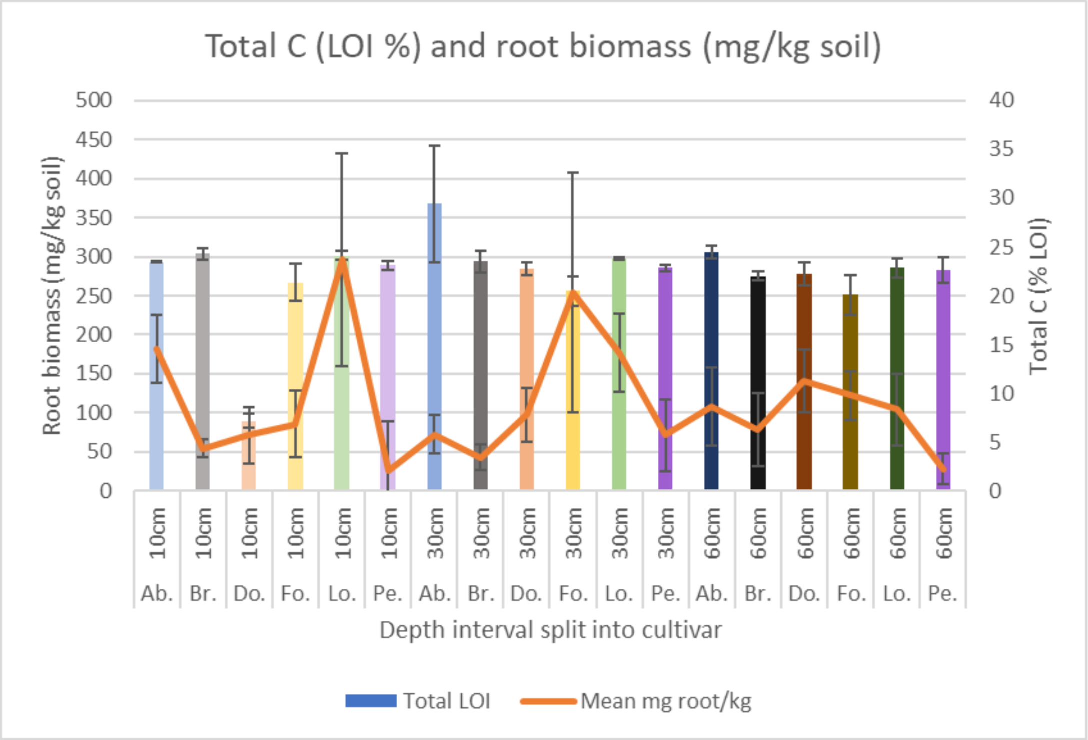


Figure 5.6 Total LOI (%) compared to root biomass (mg kg soil^{-1})

5.5.10 Patterns in root biomass and C

The result that no fraction of C is related to root biomass across any depth or any cultivar is an acceptance of the null hypothesis. A link was expected between biomass and C, given the significant differences in labile, mid-lability and total LOI soil C under different cultivars according to ANOVA and GLM. In this experiment, a major driver of this changes was expected to be root biomass across the depth profile (Pausch and Kuzyakov, 2018), with cultivars having different rooting depths as they were bred to different drought tolerances. Differences in fractions and total soil C can be driven by external inputs (Bucka *et al.*, 2019). However, there were no OM inputs on this field for two years prior to sampling, as livestock were fenced out and nothing additional was applied, so this rules out significant animal-derived OM assimilated at different speeds by separate cultivars as a source of cultivar-driven labile C turnover. Precipitation can also influence labile C through nutrient transport and processes which alter soil pH and BD, and C accumulation is greatest under water-saturated soils (DeLuca and Boisvenue, 2012). However, there were no significant differences in these variables which were identified in the GLMs as driving any C changes, so this can be ruled out. Two explanations remain: i) that time may be a significant predictor of root biomass influence on labile C, when this experiment lacks both a baseline to compare this three-year PE dataset to, and has no intervening time samples; and ii) that a main driver associated with cultivar which changes with depth has not been identified in this experiment. The likely perpetrator for a species- and depth-influencing variable as yet unidentified is soil fauna. Soil fungi, meso- and micro-fauna drive the decomposition of OM inputs in the form of litter, manure, green manure or rhizodeposition, degrading litter to secondary compounds with reduced bioavailability (DeLuca and Boisvenue, 2012). Breakdown of non-humic OM (proteins, sugars) can be very rapid while humic OM (lignin, chitin, char) are much more stable and require much longer for the soil biota to break down (DeLuca and Boisvenue, 2012). If cultivars are contributing different proportions of humic and non-humic OM to their surrounding soil, there will be a difference in breakdown time and recalcitrancies of the products. This is not reliant on root biomass or length, but on exudates and differences in tissue chemistry, which were not measured in this experiment. The microbial community can also vary between depths (Virginia, Jenkins and Jarrell, 1986) and between species or functional group (Kaštovská *et al.*, 2015), which would drive both a species and depth effect without a direct link to root biomass weight.

There is not enough evidence in this experiment to provide a recommendation of any one cultivar to have a consistent positive effect on total C or any fraction of C. Any root biomass effects on C fraction are cultivar-specific, and not a general rule of Festulolium grass cultivars. From viewing mean figures on LOI and root biomass alone, several cultivars show a response to different C storage targets: i) Fojtan is the likeliest cultivar to store C in more recalcitrant fractions; ii) Aberniche is the cultivar likeliest to store the highest proportion of total C at the deepest depth; and iii) Broadsword or Lofa the likeliest to store C in more recalcitrant fractions.

There are three strands to targeted soil C improvement: i) storing C at depth, away from environmental interference; ii) storing C in recalcitrant forms, so it is less reactive to stimuli and remains in the soil; and iii) storing more C by % weight in the soil. That different cultivars respond to each of these target strands is better evidence to support the theory of diversity in a ley, than evidence supporting any one cultivar as a panacea for carbon sequestration.

5.6 Conclusion

5.6.1 Soil parameters

No basic soil parameters (pH, BD, nutrient content, water content) were predictors of total C or any fraction of C as established by LOI (% total weight lost). This may be due to the soil condition beneath the ley, which has clayey soil subject to decades of agricultural management. Processes which change these basic soil parameters may act on timescales relevant to temporary leys, but this may be mediated by the legacy effect of previous monocultures; species-driven effects may be diluted by the fact these are cultivars of similar or crossed breeds; and there is a lack of timepoints in the experiment, which does not deliver detail on seasonal fluctuation, or accumulation of effects over time.

5.6.2 Cultivar

Cultivar was one of only two predictor variables to show a significance on total C or C fractions, the other being depth, proving the first hypothesis. Including cultivar as a predictor variable increased the proportion of data explained by the variables in the case of labile, mid-lability, and total LOI. Aberniche and Perseus drove much of the difference in labile C, while Fojtan was a more influential driver of mid-lability C, although Perseus lost the most weight at 325°C. Depth and cultivar work together to drive the influence of Fojtan on mid-lability soil C. The lack of difference between cultivars in recalcitrant C is likely because processes driving differences in recalcitrant C are likely to take place over a much longer period than the ley was established for. Cultivar, however, maintained a powerful prediction effect in total LOI, suggesting most change in total C is driven by the two more labile fractions. Only Lofa was excluded from significant in the GLM with total LOI as the response variable, with every other cultivar driving a distinct effect on total loss, the proxy for total organic carbon.

The legacy effect of previous managements may be influencing how powerful each cultivar is on current soil C processes, but without a baseline this is an unknown variable. The legacy effect of the grasses on the soil in post-ley management has also not been established. Both pre- and post-ley effects on soil C will also be dependent on many other factors including inputs, livestock, soil texture and structure, crop type and mechanical intervention.

However, this research shows that cultivar does predict labile, mid-lability and total C, supporting the hypothesis that cultivars influence belowground processes which contribute to C mineralisation.

5.6.3 Depth

Depth was a relatively weak predictor of C compared to cultivar, but did have affect total LOI and across all recalcitrancies of C. Depth explained 5.8% the variation in labile C, 5.2% the variation in mid-lability C, and 5.2% the variation in recalcitrant C, which is a consistent but very low result. Fojtan has a stronger depth effect than other cultivars in the mid-lability fraction, as its total C and proportion of that C stored at mid-lability increasing consistently with depth. Other cultivars also show difference across depth in mid-lability C, including Aberniche which stores more mid-lability C at depth than in shallow soil, and Lofa which stores less at depth. Differences are not significant between cultivars but cumulatively show a depth effect. Depth overtakes cultivar as the strongest predictor of recalcitrant C, driven mainly by differences across the depth profile of Fojtan. A relationship between all fractions of C and depth, and total C and depth, indicates a pattern in C transition with depth, related to the vulnerability of shallow soil C to environmental influences and a greater surface-derived OM input. Labile C is driven mainly by fresh OM inputs, and labile C drives much of the change in total C.

Overall, there is a pattern indicating a link between depth and recalcitrance in this experiment but it is not powerful enough to show as a significant predictor in the GLMs. This may be related to some of the limitations in this research. The null hypotheses must be accepted in this experiment regarding depth and C lability.

5.6.4 Roots

No relationship between root biomass and any fraction of C, or total C, was established. Depth was, as expected, a good predictor of root biomass, but this did not translate into a good predictor of C despite the depth-C relationship. Some patterns between root biomass and C fractions were observed in individual cultivars, but this was not detectable by the model due to large SEs and overlaps between cultivars and C values. High root biomass did not necessarily accompany high LOI values for soil C.

The lack of relationship between root biomass and C failed to provide evidence of a link between shallowness and lability, although Fojtan supported peak root biomass and the highest root:labile C ratio at 30 cm depths. While Aberniche showed a high labile C content at 30 cm, the cultivar maintained low root biomass. Mid-lability C was more variable between cultivars than labile but again, no relationships were observable with the potential exception of Lofa, which had the highest root biomass and highest LOI at 10 cm. This could suggest a relationship between shallowness, mid-lability C and root biomass in one cultivar. Fojtan again shows as a significant cultivar at 30 cm depths, with low LOI and high root biomass, but it is not a consistent pattern with a large SE overlapping with other cultivars. Fluctuating root biomass and consistent mid-lability LOI across cultivars at 60 cm produce no discernible pattern. Recalcitrant C was driven by Fojtan and Perseus, but Lofa had the highest biomass:recalcitrant C ratio at 10 cm. Broadsword, in contrast, has the second-lowest root biomass and highest recalcitrant C, negating the possibility of a general rule relating biomass to recalcitrant C content. Fojtan is the cultivar likeliest to have the greatest impact on recalcitrant C and the most likely cultivar to be driving this effect through root biomass. Total LOI reflected the lack of distinction shown across C fractions, with different cultivars producing different C-biomass relationships through the soil depth profile. However, none showed root biomass as a significant driver of these differences, and any observable patterns were cultivar-specific.

Root biomass is not a significant contributor to the C effect, and any link between subsoil biomass and C (fraction or total) is likely to be mediated by a variable missed from the GLM. This is most likely to be microbial activity, or changes in microbial community or abundance, relative to cultivar. We can reject the hypothesis that differences in SOC are driven by differences in root biomass.

5.6.5 Management implications

There is not enough evidence in this experiment to provide a recommendation of any one cultivar to have a consistent positive effect on total soil C, or any fraction of soil C. No significant relationships were established with root biomass as a driver of soil C and consequently, any species-driven differences in LOI values are being driven by an indirect effect of a variable not included in the model. Any root biomass effects on C fraction, even broad patterns, are cultivar-specific, and not a general rule across Festulolium cultivars. There are links between specific cultivars and fractions of C and total C, but without a baseline, it is impossible to tell whether they have had an overall positive effect since the ley was established. They can only be compared to each other.

There are three strands to targeted soil C improvement: i) storing C at depth, away from environmental interference; ii) storing C in recalcitrant forms, so it is less reactive to stimuli and remains in the soil; and iii) storing more C by % weight in the soil. Viewing mean figures on LOI and root biomass alone, several cultivars show a response to different C storage targets: i) Fojtan is the likeliest cultivar to store C in more recalcitrant fractions; ii) Aberniche is the cultivar likeliest to store the highest proportion of total C at the deepest depth; and iii) Broadsword or Lofa the likeliest to store C in more recalcitrant fractions.

That different cultivars respond to each of these target strands is better evidence to support the theory of diversity in a ley, than evidence supporting any one cultivar as a panacea for carbon sequestration. Therefore, the only definitive management recommendation for improving every aspect of soil C improvement is increasing diversity in grassland leys.

5.6.6 Limitations

Only one sample point was taken for this experiment, which is a significant limitation on the recommendations that can be made from the results of this experiment. Because of a lack of progression over time and the exclusion of time as a predictor variable, we are likely to be missing important details which are influential on the accumulation or degradation of fractions or total C. Seasonal variation in soil C concentration is established in the literature, and changes over WG1 and WG2 in Chapters 3 and 4 of this thesis showed significant fluctuations over time. Chapters 3 and 4 also incorporated a baseline and up to two years of data compared to one time point after four years of establishment in the Leicestershire ley.

That a relatively low proportion of data was explained by the predictor variables also indicates other important variables which were not measured in this study. One could be time. However, microbial activity is suggested as the most important driver of depth and cultivar differences in soil C, which was not measured in this experiment. This is suggested as the most likely driver given that it must be both cultivar- and depth-related, but is not necessarily directly related to root biomass.

5.6.7 Suggested future research

Future research should address the limitations discussed above. Additional timepoints and a baseline of pre-ley establishment soil, not just a control, should be incorporated into the model to show whether net gains in C and net change towards more recalcitrant forms of C are being influenced by cultivars.

Additionally, cultivar-mediated but indirect effects are driving some of the change in soil C. A key point to address in future experiments would be measuring microbial activity, either by respiration, or a measure of abundance or diversity. Future research incorporating bioinformatics could fill a crucial knowledge gap in the mediation of cultivar-driven effects on soil C.

It would also be useful in future experiments to include a greater diversity of grass species rather than only Festulolium cultivars, to see whether greater differences are driven by species, or whether cultivar is a powerful enough difference to influence long-term change.

The legacy effect of the ley would also be important to establish, because whether cultivars help to achieve an overall increase in C or not could be a very short-term effect under a change in management or land use. As land use change is a known contributor to GHG emissions by soil carbon loss, analysing the longevity of any positive C effect from a grassland ley would inform future managements and incentives to ensure the correct temporal distribution of leys for the greatest soil C benefit.

6: Meta-analysis on the effect of grazing on greenhouse gas emissions of grassland leys, and the implications for British farming in a low-carbon scenario

Abstract

Agriculture is rising to meet the challenges of food production for a growing population, while taking steps to reduce emissions due to the effects of climate change. As one of the largest land uses by area across the world, grassland for livestock grazing is an important consideration in terms of emissions reductions from the agricultural industry. Grasslands are important tools for climate mitigation, not just because of the livestock they support, but also because globally soil has become a net emitter of GHGs rather than a sink.

The UK supports a large livestock population, and while British livestock is some of the world's most efficient, emissions from grasslands supporting this livestock are often not adequately weighted in decision-making and policy, with the focus on enteric emissions. However, land use change, agricultural intensification, and the legacy of CAP mean that incorporating soil into policy can be challenging.

This meta-analysis took 59 experiments from five databases which met methodology, climate, emissions, and livestock production criteria to examine common factors influencing three of the main GHGs emitted by agriculture: CO_2, CH_4 and N_2O.

The hypotheses were:

1. Britain holds more livestock than is sustainable for maintaining soil organic carbon levels
2. Equivalent sheep LSU remains more climate-sensitive than cattle LSU
3. Upland pastures are more sensitive to changing LSU than lowland pastures
4. Intensive approaches, already inherently high climate impact, are least sensitive to increasing LSU

Generalised linear modelling (GLM) failed to detect significant predictors of N_2O from the variables identified as potential drivers across the studies. CH_4 was strongly linked to climate, precipitation, temperature, CO_2 emissions, the presence of sheep, grazing pressure by LSU, and grassland type (semi-natural, semi-improved, and improved). CO_2 responded to precipitation, climate, temperature, sheep, simultaneous cattle-sheep grazing, and CH_4 emissions.

In the UK, it is likely that reducing LSU and inputs would reduce CO_2 and CH_4 emissions. Semi-improved grassland emits higher N_2O and CH_4 emissions than improved grassland, and both are larger emitters than semi-natural grasslands. However, further research should be directed to balancing the lack of data on semi-natural systems and incorporating direct impacts of microbiota.

6.1 Introduction

Due to past carbon losses creating the potential for a soil sink, soil carbon (C) is a key consideration at a national and international scale in tackling both greenhouse gas (GHG) neutrality in the agricultural sector and in harvest sustainability. Soil carbon sequestration is currently identified as a climate mitigation tool since it has a high potential to contribute to the efforts of the agricultural sector reduce GHG emissions (FAO, 2019; Rattan Lal, 2010a; E. Milne et al., 2015). Carbon sequestration may also contribute to mitigating issues in sustainable agriculture, such as the challenges arising from the loss of C-containing compounds from the soil, including reduced nutrient availability, lowered water capture capacity, and increased erosion, which impact food supply systems and regional yield capacity (Conant and Paustian, 2002; Hirsch *et al.*, 2017; Muhammed *et al.*, 2018). The FAO estimates that significantly increasing soil organic carbon through improved land management could raise food production by 17.6 megatons per year, and help maintain productivity in the drier conditions which will become more prevalent with the ‘baked-in’ effect on climate due to GHGs which have already been released (FAO, 2019).

The increasing human population (2.5 billion in 1950 increased to 7.8 billion in 2021 (UN DESA, 2019)) places increased demand on our agricultural resources. While both population growth and calorie demand have almost plateaued (61% the world’s population now live in countries where average food consumption has reached 2700 kcal per person per day (FAO, 2015)), a slight increase in calorific demand and dietary shifts towards meat and dairy with increased economic power are still expected (Aleksandrowicz et al., 2019).

Land use change accounts for around 11% total GHG emissions globally (FAO, 2019). This is the second largest contributor after fossil fuel combustion (IPCC, 2007) and generally accompanies dietary change towards meat and dairy, and increased total food production, as forest is switched to agriculture (FAO, 2019). Carbon is lost by biomass burning and produced by livestock, especially ruminants, but there are other routes to GHG emissions: changes in C input and decomposition rates in the ecosystem; altering C returns to the soil; changes to nutrient retention; the harvesting of net primary productivity (NPP); and the physical parameters of the soil such as aeration and moisture, which influence the chemical processing of soil C, and the capacity of the soil for recovery (Poeplau et al., 2011).

Very little agricultural land is left to develop without interference; instead, under current land management schemes, agricultural productivity is prioritised to continuous cropping (Reeves, 1994), while even fallow or cover-cropped areas are part of a wider landscape under a range of pressures and are often incorporated with dual purpose into the land management of the catchment. Grazing and compaction (Baumhardt et al., 2011), tillage (Kessavalou et al., 1998), or harvesting a cover crop (hay, sileage etc.) are some of the pressures leaving soil vulnerable to erosion (Blanco-Canqui et al., 2017), which can cause reductions in soil C concentration (Kessavalou et al., 1998). Fallow areas or cover crops are generally returned to rotation before any effects of intensive agriculture degradation can be reversed, where recovery time can be on a scale of decades depending on the soil (Aguilera et al., 2013) rather than two to three years as is commonly allowed.

Managed grasslands in rotation are frequently used as one approach to dual-purpose (rotational arable and grazing) cover cropping. Grasslands are managed worldwide to support livestock over a variety of practices and intensities, from nomadic grazing to improved high-calorie pasture. The 20th century saw a significant increase in the number of ruminant livestock, from 1.4 to 3.4 billion (J. Chang et al., 2021a), reaching 4.2 billion by 2014 (Cai et al., 2017). Post-establishment, grasslands generally avoid tillage, and physical influence on nutrient and water retention and robustness to erosion are accepted benefits to establishing grasslands. ‘Grasslands’, which occur in most biomes across the globe, refer to a diverse and dynamic group of ecosystems, which range from the natural (African savanna, North American prairie, and Eurasian steppe), to the intensive anthropogenicallycreated monocultures such as *Lolium perenne* (perennial ryegrass) in Europe, or *Pennisetum clandestinum* (Kikuyu) in Australia and Asia.

Previously, natural and sparsely grazed grasslands have helped maintain a cooling effect on the planet (J. Chang et al., 2021a), as they are relatively resilient to climate change and more reliable C sinks than forests (Dangal et al., 2020). However, recent trends show a concerning transition from a net cooling to a net warming effect on climate with the increase in GHG emissions accompanying management intensification for livestock production (especially enteric CH_4 emissions, but also soil carbon and nitrous oxide flux) and the increased conversion from forest to pastureland (Chang et al., 2021a; Oenema et al., 2014; Sahoo et al., 2019). Globally, dietary changes are projected to increase cattle and sheep consumption (particularly due to dairy), resulting in increased grass biomass demand and further increases in CH_4 and N_2O emissions (J. Chang et al., 2021b).

6.1.1 UK Livestock production on grasslands

Half of the world's habitable area is used for agriculture, and 77% of that is used for grazing livestock (Ritchie, 2019). In the UK, the total utilised agricultural area (UAA) is 175,000 km^2 (DEFRA, 2020b), of which 53,630 km^2 (30.6%) was pasture and 41,570 km^2 was semi-natural grassland (23.8%, or together 54.4% all UK UAA) as of 2007 (Nafilyan, 2015). The UK is the largest producer of sheep products in the EU-28 and sustains the third largest population of cattle after France and Germany (DEFRA, 2020b), despite having a total land area of 241,930 km^2 to Germany's 349,380 km^2 and France's 547,559 km^2 (World Bank, 2020). Cattle density reflects the prioritisation in the UK of grazing agriculture, with 39.68 cattle km^{-2}, compared to France and Germany at 32.87 and 32.92 respectively (DEFRA, 2020a; World Bank, 2020).

As of June 2022, the UK livestock population is 7.04 million breeding cattle and 2.60 million calves, with sheep and lambs numbering 33.07 million (DEFRA, 2022b). There are 756,000 hectares of temporary grassland which has been established for less than five years, in addition to 3.6 million permanent hectares (DEFRA, 2021b). These grasslands produce 926,200 tons beef (National Beef Association, 2020) and 300,200 tons lamb per year (NFU, 2018). As a comparison, 2 million hectares produce 15 million tons of wheat per year, and grains provide 1/3 the average person's daily food intake (*United Kingdom Food Security Report 2021: Theme 2: UK Food Supply Sources,* 2021). The use of a potential C sink (land which could be under alternative management such as no till, no livestock, forestry etc.) as a food production system providing such a relatively small proportion of British diets is incongruent with the national and international commitments to GHG reductions targets.

6.1.2 Soil, livestock, and agricultural waste-derived GHG emissions

Common agricultural emissions gases include methane and nitrous oxide, alongside carbon dioxide. CO_2 equivalent units (CO_2-eq) are used to describe the climate impact potential through a common denominator to standardise the impact of gases over 100 years in the atmosphere, in relation to carbon dioxide. Methane (CH_4) has a CO_2-eq value of 25 times that of CO_2, or emitting one kilogram of CH_4 has the same warming potential in the atmosphere as emitting 25 kg CO_2; for N_2O, the CO_2-eq value rises to 298 times as powerful in the atmosphere for global warming potential (UNFCCC, 2007).

Generally, grasslands in agricultural control or rotation are being used to wholly or partially support livestock grazing, whereby emissions are produced from five 'pools': animals, manure, the soil, crops and feed (Schils et al., 2005). Integrated into these are any transport and storage costs, for instance of importing soy feed or storing manure. The inclusion of soil carbon sequestration or alternatively

SOC loss has a significant influence on the overall carbon budget of the farm, as does including indirect costs such as the production and shipping of imported resources consumed onsite e.g. fertiliser (Schils et al., 2005). According to 1995 data, agricultural emissions of Europe totalled 470,000,000 metric tons CO_2-eq, 11% total EU emissions, of which 56% was N_2O (produced by vehicles used in transporting inputs and outputs, operating machinery, some from manure during storage and application, and some lost from soil (Schils et al., 2005); 36% methane (produced mainly by enteric fermentation of forage, crops and grass, but some also released from manure (Schils et al., 2005); and 8% was CO_2 (produced by manure application and from the soil, but also from vehicles and machinery (Schils et al., 2005; Freibauer, 2003). Enteric emissions and manure management were responsible for 39% the total emissions (183,300,000 tons) and grasslands themselves emitted 17% the CO_2-eq (79,900,000 tons), losing carbon from soil oxidation (Freibauer, 2003). For the UK, agriculture, land use, land use change, and forestry together contributed 8% all emissions 2011 (Moran et al., 2011), but hidden in the small proportion of the emissions is the high value in global warming potential of those gases emitted – the bulk of which in agriculture is methane and N_2O, those gases with high CO_2-eq values.

Producing a kilogram of milk emits between 0.37 to 0.69 kg of CO_2 eCO_2-eq), depending on the size and efficiency of the operation (Rotz et al., 2010). A modern British dairy cow produces on average 8,152 litres of milk per year (AHDB, 2021), and milk weighs 0.97 kg per litre. Using Rotz et al (2010) units, the CO_2-eq solely of the milk yield of one British dairy cow is 2.93-5.46 tonnes per year. Given that the global population of cattle is now around a billion (Shahbandeh, 2022), the pressures of the ensuing GHG emissions on global climate is evident – billions of tonnes of carbon will enter the atmosphere from dairy production alone, even accepting that in other parts of the world, subsistence or nomadic farmers or even systems of different cattle diets and intensities, would produce differing emissions (Tongwane & Moeletsi, 2020). This is without including the land emissions of deforestation or loss of soil C from lands turned to pasture. Global whole agricultural emissions reached 9.3 bn tonnes CO_2-eq in 2018, of which 3 bn tonnes was methane and nitrous oxide from livestock, and 4 billion tonnes were released from land use change (mainly deforestation) (FAOSTAT, 2018), indirectly linked to livestock farming. Livestock supply chains account for 14.5% global anthropogenic GHG emissions (FAO, 2019), of which cattle (dairy and beef) account for around 66% (FAO, 2019). Direct livestock emissions of CH_4 and N_2O constitutes about 9% of total global emissions in CO_2-eq, and 3% of the UK's (Gill et al., 2010).

Much of the land cleared for agriculture, directly and indirectly used for livestock, will have previously been grassland of varying qualities (Johnston, 2014; Wright & Wimberly, 2013). Previously this would have supported carbon sequestration (Gilmanov et al., 2010; Lal, 2010). Through reduction and restoration, land has the potential to transform from a net contributor to emissions via agriculture, to a net sink

6.1.3 Land management influences in the UK

In the context of analysing British livestock sustainability with regards to soil carbon, it is important to understand the national and international political landscape guiding the policies that drive our food systems.

The European Union's Common Agricultural Policy (CAP), while purportedly aiming for sustainable natural resource management (European Commission, 2021a), has frequently found this aim at odds with its main focus of intensive agricultural productivity (Donald et al., 2002; Leventon et al., 2017; von Weizsäcker, 2014). CAP, supporting a rate of 33% surplus food production, takes up around 40%

the EU budget (Massot, 2021), 71% of which is spent directly on farmers' income support; therefore, *not* producing the surplus food, which has to be fertilised or fed, transported, packaged and then disposed of as waste (all emissions-heavy activities) could contribute significantly to EU GHG emissions reductions targets (European Commission, 2021a). Meanwhile, support for farmers could be retained using green subsidies, and directed to more nature-based land management. Additionally, long-term food security will rely on reducing soil degradation and C-efflux now, as referred to in the CAP reform goals published in 2021, to be adopted from 2023 (European Commission, 2021b).

This applies directly to the UK, due to the adherence to CAP until the recent British withdrawal from the EU, and the policy has a continuing relevance. The UK will continue a CAP-style subsidisation of farming to allow competitive market pricing with EU neighbours, among other reasons including environmental concern. In the UK, 'maintaining an agricultural area in a state suitable for grazing or cultivation' (InBrief, 2016) is the somewhat vague requirement for state-distributed but EU-sourced Pillar 1 direct payments (given per hectare, for owning 5+ hectare, with no other stipulations). This policy-driven approach maintains land in poor ecological condition by several routes:

- direct payment results in more productivity than is economically efficient, leading to wastefulness in terms of land use, particularly by the 13% payments currently coupled to production (DEFRA, 2013)
- eliminating direct payments altogether would lead to an immediate 6% reduction in land use (DEFRA, 2013), land which could then be set aside
- greening payments are distributed regardless of whether a clear plan is in place to channel funds into biodiversity and soil quality improvements (Schulze, 2018)
- agricultural production is still allowed even on land designated an 'Ecological Focus Area' (Schulze, 2018)
- only 5% land must be set aside even under greening payments and includes ditches, hedgerows and margins (Schulze, 2018)
- a green cover requirement was scrapped (Schulze, 2018)
- greening payments have driven improved farming practices on only 5% EU farmland (Schulze, 2018)

The UK has only half its natural biodiversity left, the lowest in the G7, largely due to agriculture-driven land use change (Davis, 2020). Therefore, the environmental steps towards restoring a carbon capture ecosystem service in the soil (Davies et al., 2016; Glukhova & Sirin, 2018; Y. Wang et al., 2021) will still be prevented by funding policy.

Additionally, and with greater historical precedent than CAP, British land management and livestock farming are linked to heritage and culture in some areas, such as the Highlands and Yorkshire dales, particularly in sheep farming. Historically, there was little other living to be made from nutrient-poor and steep gradient lands for subsistence farmers. This legacy continues, as these areas remain some of the most economically deprived in the UK (House et al., 2010). However, intensification and globalisation mean that sheep grazing on the uplands and peatlands, historically scenes of some of the greatest soil carbon changes in the UK, and which still hold up to 50% the total soil C of the UK (House et al., 2010), is no longer necessary for food production. Reassessing our ideas of what a productive landscape looks like, to account for ecosystem services including carbon sequestration and increasing soil carbon concentration, is a crucial step in redefining what constitutes a healthy and valuable landscape.

6.1.4 Grazing and soil carbon

Today, around 40% UK land area is some form of grassland; between 1990-2006, conversion from grassland to arable production (including activities such as ploughing) released 14 million tonnes of CO_2 from British soils (The Wildlife Trusts, 2021). In 2020, Britain released 505.5 million tons of GHG emissions, of which agriculture was responsible for 11% (Department for Business, Energy & Industrial Strategy, 2022). Only 2.2% British grassland remains unimproved (The Wildlife Trusts, 2021). Most British grasslands are used wholly or seasonally to graze livestock, or otherwise harvested for winter forage.

Grazing livestock in the British landscape creates an accepted but unnatural state of suppressed vegetation, as wood, wood-pasture, scrub and high forest interspersed with areas of openness and disturbance would be the most natural for most of the UK (and support higher biodiversity) (Davies & Dixon, 2012; Hodder et al., 2005). Grazing livestock in the uplands is one contributor to soil degradation by way of accelerated erosion, with a lack of a stabilising mature root network enhancing the flushing of particulate and dissolved organic C downstream (Evans, 1997; House et al., 2010). Further impacts will be seen downstream following upland sheet erosion and gullying of bare soil, as flooding is exacerbated by the deposition of substrate washed into rivers. Bare soil can be created by grazing sheep at densities as low as 0.2-0.4 sheep ha^{-1} (0.02-0.03 livestock units (LSU)) (Evans, 1997).

Livestock is generally measured in LSU, rather than head $hectare^{-1}$, as an attempt has been made to standardise the pressure different breeds of animal exert on the land they graze. For example, an ewe with lamb grazing in the uplands exerts less than a tenth of the pressure exerted by a two-year-old dairy cow on intensive lowland pasture according to the units (EuroStat, 2020). However, the development of LSU as a measure relies on the nutritional and calorific requirements of an animal according to species, breed and age, influenced by the nature of their feed (low input rough grazing compared to intensive grain feeding, for example) (EuroStat, 2020b). LSU does not take into account the full environmental pressure exerted by the animal due to other factors such as enteric emissions, the type of soil supporting the animal and related issues such as poaching, overgrazing, or water requirements. It also does not take into account the productivity or efficiency of an animal. For example, most European statistics use a value of a cow producing 3000 litres of milk annually (EuroStat, 2020a), whereas British cattle produce on average more than double this volume (AHDB, 2021). LSU remains a good and useful comparative tool for livestock burdens on the soil, but alone it is not without flaws. However, a study comparing livestock impact on soil carbon must be converted to LSU, since even an imperfect standardisation is necessary for the comparisons of methods, farm practices, and breeds which cross the UK and which are contained in the studies here analysed. Given the impact that livestock have on basic physical soil parameters such as bulk density (compaction, poaching, erosion) and organic matter content, the pressure relating to LSU will be an important factor to consider in the analysis of soil emissions and soil carbon.

Loss of soil carbon from areas under grazing pressure is viewed as a more of an indirect impact rather than a direct emission arising from the production and logistics of the farm to fork pathway. Soil C flux must be taken into account in agricultural emissions, to allow more accurate quantification of agricultural impact for policy and funding. In addition to indirect soil C loss from grasslands, direct emissions include GHGs emitted from farms composed of: CO_2 (respiration, transport, soil); ii) CH_4 (majority enteric emissions); and iii) N_2O (majority from application, leaching and run-off of fertiliser). Measuring these across a whole farm is difficult, relatively inaccurate, and expensive (Rotz et al., 2010), requiring different specialist equipment for each GHG measured for each individual source/sink (McGinn et al., 2014; Soussana et al., 2007). To get an accurate picture, all emissions must be accounted for, but this much time, equipment and money are rarely invested in this aspect of soil science for agriculture; additionally, the unenclosed nature of the sample sites and subject animals leaves uncontrolled variables for which even the most rigorous of studies struggle to account. The differences from farm to landscape scale particularly influences the emissions of N_2O, heavily dependent on weather, water table, river catchment, vegetation to absorb run-off (for fertilizer run-off and microbial mobilization), and in animal density and the C:N ratio of consumed vegetation (influencing urea content and density of excreta patches) (Benckiser et al., 2015; Cai et al., 2017; De Klein & Ledgard, 2005). Emissions are also dependent upon end products, location, and individual farm management, so large differences can occur between farms (Rotz et al., 2010), let alone mapping across an entire country or even continent.

Taking soil carbon into account on grasslands, which contribute around half of all UAA in the UK, is as important as enteric or fertiliser-derived farm emissions for a holistic picture of where agriculture is contributing to the GHG problem, and how it could be utilised as part of a wider solution. As stated above, the figure of 17% UK emissions originating from soil is a significant figure, to not just halt decline, but also to utilise the C storage potential left behind and turn soil from a source to a sink of C. The double threat of food supply sustainability and climate must be addressed by prioritising demands and efficiency to best target optimal outcomes and ensure climate, soil and harvest sustainability.

Considering the impact of livestock on agricultural systems this paper uses a meta-analytical approach to answer the questions: how much livestock is sustainable per unit area in the UK, in terms of stated climate targets?

32 papers from studies on soil emissions from grasslands were analysed in terms of 59 individual experiments contained therein. The studies were focused on Britain, or in climates or production systems comparable to Britain (e.g. arid, semi-arid, and permafrost were excluded, as were subsistence or nomadic grazing). This study takes into account direct and indirect emissions of carbon dioxide, methane, and nitrous oxide from these agricultural sources, with particular attention to intensity and location of grassland pasture, soil-based processes, and LSU.

Hypotheses

5. Britain holds more livestock than is sustainable for maintaining soil organic carbon levels
6. Equivalent sheep LSU remains more climate-sensitive than cattle LSU
7. Upland pastures are more sensitive to changing LSU than lowland pastures
8. Intensive approaches, already inherently high climate impact, are least sensitive to increasing LSU

6.2 Methods

6.2.1 Data collection

A meta-analysis was conducted in April 2022 using four science paper databases: BioOne, PubMed, Web of Science (WoS), and Scopus.

The databases were searched for studies providing results from grasslands on N_2O, CH_4, and CO_2 emissions. They could be from grazed or ungrazed grasslands, describe enteric emissions or not, use sheep or cattle (or none) as the grazing animals, account for one or more of the gases, and grass could comprise whole or part of the livestock diet. The design allowed for data gaps in papers because very few studies would provide data on the whole range of variables that we wanted to compare in this review.

Google Scholar was later accessed because of the low initial return rate on relevant papers, and to fill in gaps left by the more genre-specific databases. Searches extended to the year 2000 when possible.

A variety of methods were included because the study was not focused on the type of methodology, but on the gaseous emissions results compared to livestock load. However, a primary study is preferable to a model as it is subject to real-life fluctuations due to uncontrolled variables. There is also a potential issue in comparing models to primary studies, as models may have used data from the primary studies included in the analysis. As far as possible, this was checked, and additionally double-counting is unlikely to be an issue in this analysis as the range of variables here analysed frequently don't overlap in papers, and if some overlap has occurred, the mean datapoints are fed into a model where we account for frequency and look for Additionally, there are common established methods which consistently reappeared in the literature including static chambers and eddy covariance. Methods outside of this were not necessarily discounted.

Appendix 1 contains the full list of papers returned by each search, the database(s) they were returned in, the stage at which they were excluded, and the reasons for inclusion/exclusion.

The full list of search terms is explained in Fig. 6.1.

Of the papers returned using the search terms, the criteria for inclusion into this meta-analysis were:

1. Location: a climate and forage plant/forage community comparable to that commonly practiced in the UK
2. Livestock production method: an upland or lowland, intensive or extensive system of grazing with livestock commonly found in the UK, and adhering to practices commonly used in UK livestock production
3. Study type: primary data was prioritised and studies had to account for at least one soil GHG emission. Modelling was included if all the primary data and climatic factors feeding the model remained relevant to the UK system (every study within adhered to all other inclusion criteria)
4. Robust methodology: consistent with other literature, of an appropriate timescale, accounting for variables such as wind speed and direction, precipitation, temperature, and livestock loading. Replicable and using accepted methods.
5. No obvious conflict of interest or bias from the author, publisher, funding body or related organisations
6. Study duration: over one month to take account of weather differences and most studies took place over a much longer timescale to take account of climatic/seasonal variation

Exclusion criteria:

1. Location: a climate and/or forage which is not commonly found in the UK, such as aridity, desertification, or prairie
2. Livestock production method: a livestock production method not applicable to the processes which would affect GHG emissions from UK agriculture such as nomadic herding, steppe or mountain farming, subsistence agriculture, or mega scale ranching. (Of over 192,000 farms

in the UK (Norton, 2019), only 1,099, or 0.57%, are ‘mega-farms’ of over 700 dairy or 1000 beef cattle (Wasley et al., 2017) and the largest farm in the UK houses 3,000 cattle (Wasley et al., 2017); therefore ranching of 10,000 cattle or more, common in the Americas and Australia, would be incomparable.)

3. Reviews were excluded on the basis that they take a much broader perspective on a continental or even global scale, and there was no feasible way to vet the methods and data that went into every reviewed paper to ensure they adhered to the other criteria
4. Methodology had to incorporate taking the GHG data required for this review. For example, if the chamber method was used, but for only excreta patches, the study could be excluded for its omissions; if the data was not primary or followed global trends, it was excluded; if the methods were designed in such as way as to mitigate gas contribution rather than measure it, it was excluded (see Appendix 1). The methods also had to be rigorous (replicable, with standards or controls) and use methods common in the literature, such as collars and chambers, sonic anemometers, and open path lasers.
5. If an obvious conflict of interest was detected, e.g. being published by the American Meat Science Association (a body with links to meat production).
6. Studies which took place over less than one month were determined to exclude weather variation when rainfall and ground moisture are known influences on CO_2, N_2O and CH_4 emissions from the soil. Therefore they were excluded from this review (with one exception, see Appendix 1).

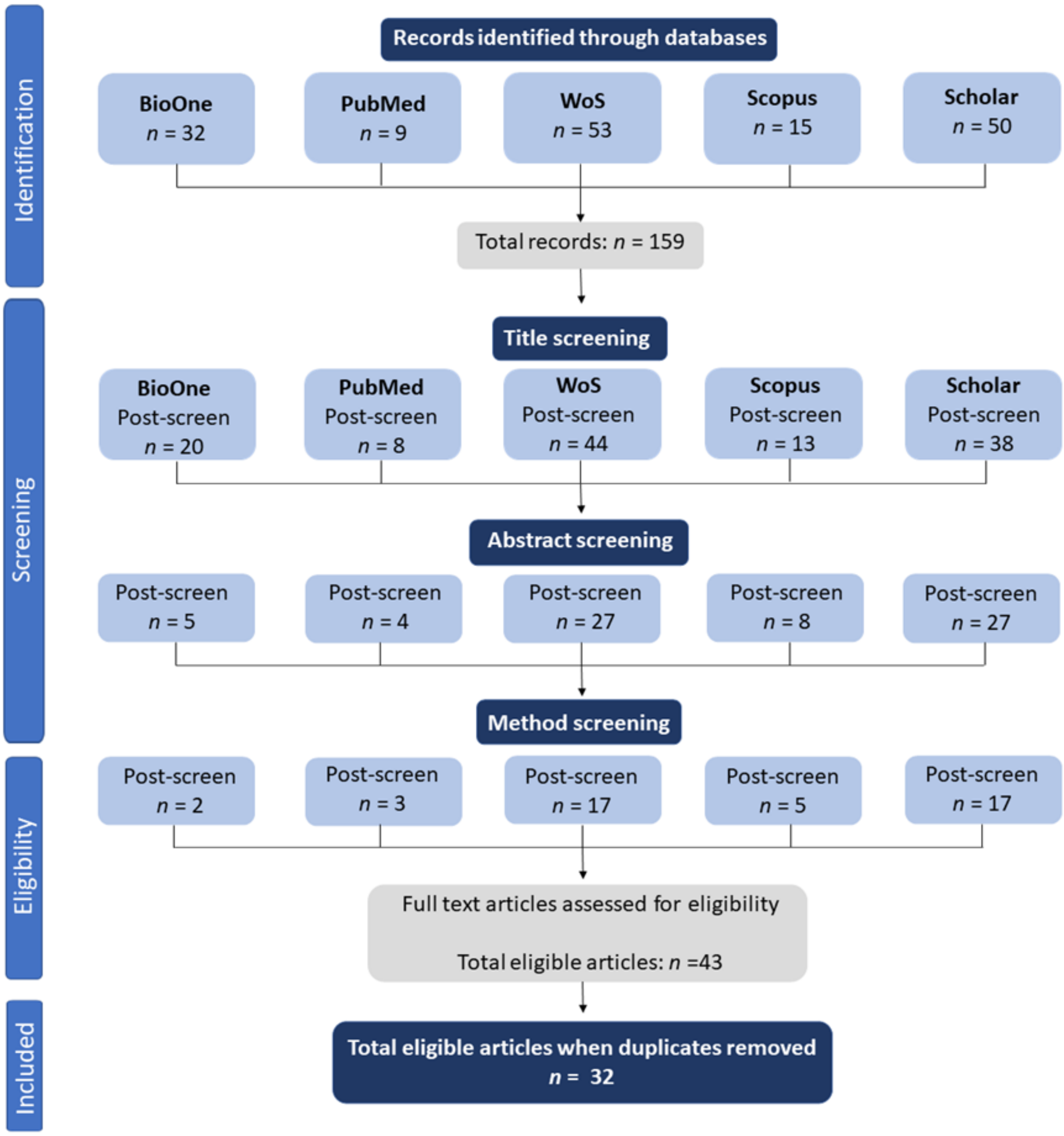


Figure 6.1. Flow chart showing the number of papers eliminated by each stage from each database according to PRISMA guidelines

The largest elimination factors following the initial key-word search were due to a lack of UK-biome relevance (e.g. grasslands within arid environments) and/or not containing appropriate primary research (e.g. a focus on grassland diversity) or the relevant modelling criteria (e.g. a model focused on mitigation for policy application).

Initially, BioOne, PubMed, Web of Science (WoS) and Scopus databases were searched for papers published between 2000-2022 (PubMed reduced to 2010-2022 because of a restricted search function) between 7th February - 8th April 2022. The keywords ‘soil, carbon, grazing, pasture, carbon dioxide, methane, N_2O, nitrous oxide, CH_4’.

A later search in Google Scholar was performed in April 2022 due to the limited number and relevance of initial results. In addition to the terms used in the first and second search cycles, ‘emission, balance, enteric, grassland, herd’ were added, producing a total of 15 eligible results.

The entire five-database search produced 32 suitable papers, the databases of which and the stages of elimination are illustrated in Fig. 6.1. The total list arising from initial search results can be found in Table 1 in Appendix 1, with justification for inclusion or exclusion. Table 6.4 in the results section lists the papers selected for inclusion with the accompanying data used in this review.

Using definitions taken from Champion (2022) (Table 6.1), I categorised the studies into comparable forms of UK grassland. I have distinguished between semi-natural lowland and upland grasslands, as lowland grasslands are generally comprised of richer or deeper soil, and uplands often characterised by stonier, less fertile soil or more heavily eroded soils which have been historically used for only sheep on 'rough grazing'. There is no official definition, however a distinction can generally be drawn (DEFRA, 2010).

Table 6.1. Definition of UK grassland categories within this chapter

Grassland	**Definition** (Champion, 2022)
Natural	Grassland that is the climax vegetation, i.e. where the climate or natural conditions prevents scrub or trees establishing. Unmanaged by human activity. Very rare in the UK and, by definition, not found on any land used for grazing or productivity.
Semi-natural lowland	Grassland where cutting, grazing or burning prevents scrub or trees becoming established, but is otherwise unaltered by human activity such as drainage or fertiliser application. Generally more fertile than semi-natural upland, with a range of uses.
Semi-natural upland	Grassland where cutting, grazing or burning prevents scrub or trees becoming established, but is otherwise unaltered by human activity such as drainage or fertiliser application. Generally less fertile than semi-natural lowland with historical rough grazing, sheep-dominated.
Semi-improved	Grassland that has had some agricultural improvements made to it, such as drainage or some fertilisation, but where botanical biodiversity is maintained through a mixed sward.
Improved	Grassland that has been managed to increase its productivity, usually by a combination of drainage, fertilisation, herbicide use, ploughing and/or reseeding.

Livestock units (LSU) transform livestock numbers to reflect the pressure exerted by the species of livestock on a pasture – sheep exert less grazing pressure per head than cattle etc. I have used the LSU guidance from the Rural Payments Agency (2021) (Table 6.2). All livestock numbers were converted from the given units (for example, in head per hectare or in Australian LSU) into European LSU.

Table 6.2. LSU official guidance

Livestock	**LSU**
Cattle over 2 years	1
Cattle 6 months – 2 years	0.6
Lowland ewe with lamb	0.12
Ram	0.12
Store lamb	0.08
Upland ewe with lamb	0.08

As there is no official guidance on the LSU which defines intensive or extensive grazing, I have determined categorisation by following guideline stocking rates for the UK in the conservation of grassland report from the Scottish Agricultural College (2007) (Table 6.3). The recommended LSU value of <0.25 ha^{-1} y^{-1} to maintain biodiversity, referenced in Table 6.3, originates from Scottish Agricultural College (2007) guidance.

Table 6.3. Definition of extensive and intensive grazing in LSU, according to grassland type as defined in Table 6.1.

Type of grassland	**LSU ha^{-1} y^{-1} - extensive**	**LSU ha^{-1} y^{-1} – intensive**
Natural	<0.25	N/A
Semi-natural lowland	<0.40	>0.41
Semi-natural upland	<0.25	>0.26
Semi-improved	<0.60	>0.61
Improved	<1	>1.1

Data taken from each paper had to be congruent in terms of units and able to be categorised with the most relevant type of UK grassland; methods were generally chambers and eddy covariance, although, as mentioned, some models where each paper met the conditions of location, production, methodology and bias were included. A significant deviation from UK climate generally excluded a paper from this review, however, with a dearth of data on sheep grazing, some adjustments had to be made to include more Mediterranean climates. Climate category was ranked in comparability to British climate to account for this, with climate ranking used alongside the proxy values of temperature and rainfall to be incorporated into the model as predictor variables. 1 = British climate, with 2 as a comparable climate (slightly warmer, wetter or drier, but broadly comparable – for instance New Zealand or Germany), and 3 as a Mediterranean climate. Irrigation is, however, often a factor in studies undertaken in drier climates, and so the effect of water content in the soil on gaseous emissions may be mediated to some extent. All gaseous emissions were transformed from original units as published to common units of emissions in g m^{-2} year^{-1} (Table 6.4). Table 2 in Appendix 1 contains supplementary information for the calculations used to convert data from studies into common units for this research. Enteric emissions were published using a wide range of units across papers and these were transformed to give a standard unit of grams of emission per kilogram of liveweight (as opposed to per head or per carcass) per year. Details are given in Table 6.4 below, with values for the predictor and response variables in the common units rather than raw data.

Table 6.4. Papers categorised by grassland and grazing extent, listing predictor and response variables run in the LMs and GLMs

Reference	Experiment	Climate	Precip_mm	Temp_C	Livestock	LSU	Grazing_type	Grassland_Type	Experiment_duration_d	Soil_N2O_g_m2_y	soil_N2O_CO2_eq	Soil_CH4_g_m2_y	soil_CH4_CO2_eq	Soil_CO2_g_m2_y	whole_soil_flux_g_CO2_eq_m2_y	Enteric_g_CH4_kgLW_d	Enteric_g_CO2_eq_kg_LW_d
Allard et al 2007	1	2	1200	7	Cattle	1.03	Int	SI	1095	0.058	17.284	61.2	1530		-97	0.56	14
Allard et al 2007	2	2	1200	7	Cattle	0.5	Int	SI	1095	0.013	3.874	31.6	790		-75	0.56	14
Beauchemin et al 2010	NA	3	277		Cattle	0.32	Int	I	2920							0.063	1.577
Beauchemin et al 2011	NA	3	277		Cattle		Int	I	2920						-0.002	0.002	0.044
Boon et al 2014	1	1	1005	10	None		Nor	SI	56			-0.0892	-2.232	191.99			
Boon et al 2014	2	1	1005	10	Cattle	0.27	Ext	SI	56			0.0035	0.088	273.84			
Charteris et al 2021	1	1			Sheep	0.39	Ext	SNU				0.0144	0.3606	273.66			
Charteris et al 2021	2	1			Sheep	2.9	Int	I				0.005	0.1256	5990.1			
Chiavegato et al 2015	NA	2	828	17	Cattle	1.6	Int	SI	1095			3E-05	0.0007	5441.5			
De la motte et al 2019	NA	2			Cattle	1.5	Int	I	1095						940.5		
Dengel et al 2011	NA	1			Sheep		Int	I	2920			0.0405	1.0119	1377.6			
Drewer et al 2017	1	1	958	8.8	Sheep	0.84	Ext	I	730	10.32	3076.6	2.336	58.4	8030			
Drewer et al 2017	2	1	958	8.8	Sheep	0.84	Int	SI	730	0.069	20.511	0.584	14.6	8030			
Du mortier et al 2017	NA	2	508		Cattle	2.3	Int	I	547.5			10	250			0.153	3.829
Flessa et al 2022	NA	2	833	7.4	Cattle	0.98	Int	SNL	2190	0.346	103.02	-0.094	-2.353			0.424	10.59
Hammar et al 2002	NA	2	626	6.8	Cattle	1.5	Int	I	3650					2			
Hortnagl et al 2018	1	2	1682	6.1	Cattle		Int	I	365			-0.08	-2	-2806			
Hortnagl et al 2018	2	2	1682	6.1	Cattle		Ext	SI	365			-0.16	-4	-1371			
Hortnagl et al 2018	3	2	852	6.5	None		Nor	I	365			0.56	14	-111			
Hortnagl et al 2018	4	2	933	8.6	None		Nor	I	365			0.36	9	-737			
Hortnagl et al 2018	5	2	1151	9.1	None		Nor	I	365			-0.08	-2	-2403			
Hortnagl et al 2018	6	2	1151	9.1	Cattle		Int	I	365			-0.08	-2	-912			
Hortnagl et al 2018	7	2	1151	9.1	Sheep		Int	I	365			1.72	43	-1246			
Huth et al 2012	NA	2	711	9	Cattle		Ext	SI	730	2.396	713.97	0.3504	8.76	1.614			
Jerome et al 2014	NA	2	572	9.5	Cattle	2	Ext	SI	730					-			
laubach and hunt 2018	NA	2	839		Cattle	2.36	Ext	I	1095	1.069	318.49	4.92	123	295.53		0.833	20.82
Laubach et al 2016	1	2	839		None		Nor	SI	730	0.555	165.45	4.1485	103.71				
Laubach et al 2016	2	2	839		None		Nor	I	730	0.791	235.76	3.5414	88.535				
Lawton 2005	1	2	1470	8	Cattle	2.2	Int	I	1095	0.76	226.48						
Lawton 2005	2	2	1470	8	Cattle	2.2	Int	I	1095	0.85	253.3					0.548	13.7
Liang et al 2020	1	3			Cattle		Int	I								0.339	8.479
Liang et al 2020	2	3			Cattle		Int	I								0.401	10.03
Liebig et al 2010	1	3	410	4	Cattle	1.38	Int	I	1	0.471	140.29	-0.278	-6.95		-203	0.037	0.983
Liebig et al 2010	2	3	410	4	Cattle	0.23	Ext	SNL	1	0.187	55.79	-0.293	-7.329		-169	0.006	0.155
Liebig et al 2010	3	3	410	4	Cattle	0.66	Ext	SNL	1	0.2	59.482	-0.308	-7.709		-180	0.049	1.23
Manono 2016	1	2	600	12	Both	3.3	Ext	I	17	0	0	0	0	0			
Manono 2016	2	2	600	12	Both	3.3	Ext	SI	17	-0.04	-11.53	0.0011	0.0285	0.5588			
Manono 2016	3	2	600	12	Both	3.3	Ext	I	17	-0.02	-5.477	-0.002	-0.057	0.9021			
Manono 2016	4	2	600	12	Both	3.3	Ext	I	17	-0.02	-4.763	-0.002	-0.057	0.8973			
McGinn et al 2014	1	3	353	4.7	Cattle	0.1	Int	SNL	730							0.43	10.74
McGinn et al 2014	2	3	353	4.7	Cattle	0.2	Int	SNL	730							0.43	10.74
Samsonstuen et al 2020	1	3			Cattle	0.78	Ext	SI	1460	0.323	96.369					0.993	24.84
Samsonstuen et al 2020	2	3			Cattle	0.78	Ext	SI	1460	0.339	101.01					1.043	26.08
Samsonstuen et al 2020	3	3			Cattle	0.78	Ext	SI	1460	0.348	103.74					0.94	23.51
Schaufler et al 2010	1	2	500	9	Cattle	0.75	Ext	SNL	1	2.917	869.25	0.0035	0.0876				
Schaufler et al 2010	2	1	850	9	Sheep	1.55	Ext	I	1	7.076	2108.5	0.0152	0.3796	8566.4			
Schaufler et al 2010	3	2	1100	11	None		Nor	SNL	1	0.595	177.21	0.0292	0.73	3353.3			
Schils et al 2013	1	1			Cattle		Int	SI	365	0.227	67.679						
Schils et al 2013	2	1			Sheep		Nor	SI	365	0.227	67.679						
Skiba et al 2013	1	1			Sheep	0.2	Ext	SNL	1095	0.003	0.8				267	0.038	0.959
Skiba et al 2013	2	1			Sheep	0.77	Ext	I	1095	1.289	384				1576	0.316	7.898
Soussana et al 2004	1	2			None		Nor	I	3650						20		
Soussana et al 2004	2	2			None		Nor	I	3650						-40		
Stewart et al 2014	1	3	506	3.5	Cattle	0.08	Ext	I	82			0.2112	5.28			1.786	44.66
Stewart et al 2014	2	3	506	3.5	Cattle	0.33	Ext	I	82			0.4652	11.63			1.531	38.26
Stewart et al 2014	3	3	506	3.5	Cattle	0.32	Ext	I	82			0.3656	9.14			1.621	40.52
Thomas et al 2012	NA	3	600		Cattle	1.43	Int	I	40150					-179		0.798	19.95
Thomas et al 2017	1	3	319	13	Cattle	0.7	Int	SNL	1460			0.3833	9.5813	0.0061			
Thomas et al 2017	2	3	319	13	Cattle		Nor	SNL	1460			0.3833	9.5813	996.45			
Wu et al 2022	1	1	1030	10	Cattle		Int	I	2555	0.319	94.934	12.275	306.88	561.23		0.472	11.81
Wu et al 2022	2	1	1030	10	Sheep		Int	I	2555	0.319	94.934	12.275	306.88	561.23		0.352	8.802

6.2.2 Analysis

Four generalised linear models were run with soil N_2O flux in g m^{-2} y^{-1}, soil CH_4 flux in g m^{-2} y^{-1}, soil CO2 flux in g m^{-2} y^{-1}, and enteric CH_4 emissions in g CH_4 kg liveweight^{-1} d^{-1} as response variables. The predictor variables were climate, livestock type, grazing type and grassland type (categorical) and precipitation and temperature (continuous).

6.3 Results

6.3.1 Studies

The 32 articles from 5 databases, split into 57 separate experiments, were grouped into 3 climatic zones. 1 is a UK climate; 2 is comparable to UK climate but with some differences, e.g. slightly colder average temperatures or slightly increased annual precipitation, such as France, Germany, Norway or New Zealand; and 3 being a Mediterranean climate. I would have preferred to exclude these Mediterranean climate studies, but due to a significant shortage of temperate research on sheep grazing, it was necessary to widen the climatic envelope to areas which do support high levels of sheep grazing such as parts of Australia.

The papers were divided into those with and without livestock, and of those with, whether they were grazed by sheep or cows or both. Grasslands were also divided into intensive and extensive grazing as per the definitions in Tables 6.1 and 6.3. They were also defined as semi-natural upland or lowland, semi-improved, or improved in condition with reference to Table 6.1.

Table 6.5 below details the number of papers in each category. Because of their prevalence in the landscape, the number of papers studying improved intensive cattle grazed grasslands outstrips the other categories considerably.

Table 6.5 Number of experiments in each category according to livestock, livestock density, and grassland type.

	No livestock	**Sheep**		**Cattle**		**Both**	
Grassland type	**N/A**	**Extensive**	**Intensive**	**Extensive**	**Intensive**	**Extensive**	**Intensive**
Semi-natural upland	0	2	0	2	1	0	0
Semi-natural lowland	3	0	0	1	3	0	0
Semi-improved	2	0	1	3	8	0	1
Improved	6	2	5	3	15	0	3

Of these 57 experiments, 11 had livestock present but no verifiable values for LSU. There were 37 experiments with cattle and 17 with sheep (including five experiments which utilised both cattle and sheep grazing). Of 25 studies which included enteric emissions data as well as soil flux, 20 were on cattle enteric emissions and five on sheep enteric emissions. Four experiments were carried out on semi-natural uplands, eight on semi-natural lowlands, 11 on semi-improved grassland, and 34 on improved grassland. Of the experiments, 34 involved intensive grazing and 13 on extensive grazing, and seven without livestock (Table 6.5).

For the more specific conditions, only one experiment each was found to cover semi-natural upland extensive cattle grazing; semi-natural upland extensive sheep grazing; semi-natural upland intensive cattle grazing; semi-natural upland intensive sheep grazing; semi-improved intensive sheep grazing; and semi-improved without grazing (Table 6.5). Two experiments (from the same paper) also specified that they took place on peatland, which has not been used as a separate category in this review as it overlaps with management descriptors and/or grazing intensity categorisation. Two experiments each were found for the conditions of semi-improved extensive cattle; semi-natural lowland without grazing; and semi-improved intensive grazing by both sheep and cattle. Three experiments each adhered to the conditions of semi-natural lowland extensive cattle; semi-natural lowland intensive cattle grazing; improved extensive cattle grazing; improved extensive sheep grazing; and semi-improved intensive grazing by both sheep and cattle. Five studies investigated semi-improved intensive cattle grazing. Six experiments addressed both improved grassland without livestock, and improved intensive sheep grazing. And finally, 15 of the experiments investigated improved intensive cattle grazing. No experiments were found to cover the conditions of semi-natural lowland or semi-improved extensive sheep grazing. Likewise, there were no experiments including both sheep and cattle on any semi-natural grasslands or in extensive grazing systems.

6.3.2 Modelling

Soil N_2O emissions

Deviance residuals: 1Q = -0.5025, median = -0.3780, 3Q = 0.8918. Not even around the median, but close to 0.

A generalised linear model (GLM) for the response variable of soil N_2O in g m^{-2} y^{-1} produced no indication that any of the predictors were statistically significant drivers. Categorical variables (climate, livestock type, grassland type, grazing type) were all coded as such.

The predictors fed into the model were climate, precipitation, temperature, livestock type (whether cattle, sheep, both or none), LSU, grazing type (intensive or extensive) or grassland type (improved, semi-improved, semi-natural upland or semi-natural lowland).

There were not enough datapoints in the adjacent CO_2 and CH_4 columns to run a second GLM including other soil gas emissions, given that many papers focused on one or two GHGs.

Soil CH_4 emissions

GLM showed that climate has a significant impact on soil CH_4 emissions. The predictors fed into the model were climate, precipitation, temperature, soil CO_2 emissions, livestock type (whether cattle, sheep, both or none), LSU, grazing type (intensive or extensive) or grassland type (improved, semi-improved, semi-natural upland or semi-natural lowland).

Deviance residuals: 1Q = -1.030, median = -0.234, 3Q = 1.570, median close to 0 and residuals fairly symmetrical.

Several other factors also showed this high level of significance:

- UK-adjacent climate (i.e., temperate maritime climate in northern Europe or New Zealand): $p = 0.006$
- Mediterranean climate is a very strong predictor: $p = 0.005$.
- Precipitation (mm): $p = 0.009$
- Temperature (°C): p = 0.003
- Soil CO_2 emissions (g m^{-2} y^{-1}): $p = 0.003$
- Sheep grazing: $p = 0.003$
- Semi-natural lowland grassland: $p = 0.001$
- Semi-improved grassland: $p = 0.001$
- Improved grassland: $p = 0.001$
- LSU: $p = 0.011$

Soil CO_2 emissions

GLM showed several significant predictors of soil CO_2 flux. All of the variables listed below had a *p* value of <0.01

- Precipitation (mm)
- UK-adjacent climate
- Mediterranean climate
- Temperature (°C)
- Sheep as the grazing livestock
- The presence of both sheep and cattle as grazing livestock
- CH_4 emissions (g m^{-2} y^{-1})

Both CO_2 and CH_4 models failed to run with N_2O as a predictor, probably because there weren't enough datapoints for N_2O to compare to all other variables.

6.3.2 Common variables occurring with GHG soil sequestration effect

All studies with a loss of CO_2, CH_4 or N_2O were removed, to observe common characteristics of grasslands with a net gain of each GHG (minus figures describe sequestration into the soil, and positive figures describe a net loss to the atmosphere).

Soil N_2O sequestration

Only three experiments out of 59 had a negative N_2O value, and all were from the same paper (Manono, 2016). All three had the same climate (temperate maritime, non-UK), average annual precipitation (600 mm), mean annual temperature (11.7°C), livestock (both sheep and cattle), and grazing type (intensive). One experiment took place on semi-improved grassland and two on improved.

The LSU was also the same across the all experiments, and, at 3.3, is particularly high, and not representative of all studies. An LSU of 3.3 is triple the requirement for an 'intensive' designation even on improved grassland.

However, a mean net N_2O sequestration of -0.024±0.007 g m^{-2} y^{-1} was achieved.

The current area of grassland in the UK is 5.6 million hectares. If every ha of grassland in the UK was improved and grazing pressure set uniformly at 3.3 ha, 18.4 million cattle at 1 LSU each could be supported (compared to a total current population of 9.63 million cattle and 15.78 million adult female breeding sheep, a combined total of around 12.92 million LSU).

However, currently only 56.33% UK grassland supporting livestock is improved (Nafilyan, 2015) and 43.67% is semi-improved or semi-natural, meaning it should support a maximum of 1 LSU under current guidelines. This produces a potential population of 10.4 million cattle on improved grassland at 3.3 LSU, and an additional 2.4 million cattle on all other grassland at 1 LSU – a total population of 12.3 million LSU (one cow = 1 LSU). This is very close to the existing population of livestock.

Soil CH_4 sequestration

Following the same process as above, retaining only experiments which resulted in a net gain of soil CH_4 produced 11 results.

66.63% of these were in UK-comparable climates and one study (9.09%) was British. Therefore 72.73% the studies are in temperate maritime climates, with a mean precipitation of 1088 mm and a mean annual temperature of 8.9°C.

27.27% the studies originated from Mediterranean climates and were all from the same paper (Liebig et al., 2010). These had a much lower annual precipitation rate (410 mm) and due to a winter study, also a lower mean temperature recorded in the paper (4°C) which covered the duration of the experiment and was not an annual mean.

The mean LSU over the six studies with a recorded value was 1.64±0.55. Two of the studies had no grazing (18.18%) and three grazed sites had no recorded LSU value so were removed from the mean/SE calculations.

63.64% the sites were cattle-grazed and 18.18% grazed by both sheep and cattle. None were grazed by only sheep.

54.55% the sites were on improved grassland, 18.18% on semi-improved, and 27.27% on semi-natural lowlands.

66.66% the studies were intensively grazed and 33.33% extensively grazed.

Assuming a mean LSU of 1.64 across all grassland in the UK, this would support 9.2 million LSU (equivalent to 9.2 million cattle or 57.4 million lowland ewes). However, only 56.33% the 5.6 million hectares of UK grassland is improved, producing a potential value of 2.6 million LSU at 1 LSU ha^{-1} on semi-improved grassland and 5.2 million LSU at 1.64 LSU ha^{-1} on improved grassland. This totals 7.7 million cattle or 48.4 million sheep.

Soil CO_2 sequestration

Following the same process as above, retaining only experiments which resulted in a net gain of soil CO_2 produced eight results, of which seven have a similar climate to the UK and one has a Mediterranean climate.

Mean annual precipitation is 1150.5 mm and mean annual temperature 7.8°C.

37.5% the studies showing carbon sequestration had no grazing at all, despite making up only 15.25% the total number of studies including net C loss.

50.00% the sites were cattle-grazed and 12.50% grazed sheep. However, only one study had an LSU value available. This was 1.43 and it is for the study in the Mediterranean climate, cattle-grazed on intensive improved grassland.

Seven out of eight sites were on improved grassland and one on semi-improved.

Of the five studies which included grazing, four were intensively grazed and these were all also improved.

Assuming a the LSU value of 1.43 across all grassland in the UK, this would support 8.0 million LSU (equivalent to 8.0 million cattle or 50.0 million lowland ewes). However, only 56.33% the 5.6 million hectares of UK grassland is improved, producing a potential value of 2.0 million LSU at 1 LSU ha^{-1} on semi-improved grassland and 4.5 million LSU at 1.43 LSU ha^{-1} on improved grassland. This totals 6.5 million cattle or 40.7 million sheep.

6.4 Discussion

6.4.1 Land use context

It is important to read these results in the context of the scale of the land uses analysed. In the UK, there are 5.6 million ha intensive improved pasture as of 2007, which is 22% the area of the UK (Nafilyan, 2015). Temporary grassland, which is very under-researched given its prevalence across the world, occupies 1.22 million ha, of which grassland less than five years old accounts for 98.4% (DEFRA, 2020b).

Semi-improved (cut, drained, burned and otherwise managed) peatland occupies 1.23 million ha, which is 41% the total UK peat area (UK Centre for Ecology & Hydrology, 2020). Peat was not a separate category in this study, as it was generally not specified whether the studies in this meta-analysis occurred on peat or not. However, because of the fertility of peat soil, their distribution and sheer acreage across the country, a large proportion of peatland is likely to be farmed. Exact figures are currently being investigated using the England Peat Mapping strategy, which became active in April 2021 (Evans and Raven, 2017). Because of the high C-content of peat soil, the consequences of intensifying production and utilising peat for grazing are also likely to be disproportionately higher than for the average grassland with low-C soil in this meta-analysis. This is an important consideration in a country like the UK with at-risk peat soils, and a large potential source of emissions under common agricultural land management strategies. Peat was recently considered a carbon sink, but when soil emissions are included in GHG accounting, it turns from a sink to a source, with 11 million tons of CO_2-eq released by peatlands each year (Evans and Raven, 2017). This is mainly due to agriculturally driven land use change (Poppe, van Duinen and de Koeijer, 2021), hence current efforts to map the peat, in order to protect it.

The scale of grassland cover, including on high-C soils vulnerable to change, means that any small fluctuations in soil emissions with land use observed on one farm or in one study will be proportional when extrapolated to a UK-wide scale. Given that the range of studies in the meta-analysis are limited, particularly by a lack of upland or sheep-centred emissions, insignificant but observable patterns in the meta-analysis could scale up to have a disproportionate impact to that indicated in this research.

Further context for interpreting the results of this meta-analysis comes from the populations of livestock currently supported in the UK. 9.6 million cattle, 16.6 million sheep and 16.7 million lambs currently call the UK home (DEFRA, 2022b). Again, extrapolating the impacts across one farm to the whole UK using those livestock figures provides a perspective on the scale of the issue of the impacts of livestock farming on soil GHG emissions, especially when figures are conducted in LSU.

If all UK livestock were supported solely by UK grassland (on 2007 figures of 5.6 million ha), we would be supporting an LSU of 12.96 million over all grassland or a total LSU of 2.31 ha^{-1}. This is double the highest LSU threshold for defining 'intensive' grazing, which is on improved grassland (1.1 LSU). As it is, most livestock is reared partially on grasslands and partially in feedlots, consuming grain rather than entirely grass/herb pasture; grain which has a higher productivity rate and calorie content than grass, but lower nutrients and higher input demand (Pimentel *et al.*, 1980). Much of this is also imported, with associated environmental impacts of lower regulation overseas, and less control on inputs or land use change (McAlpine *et al.*, 2009), more fossil fuel requirement in terms of production and transport (Lehuger, Gabrielle and Gagnaire, 2009). Agricultural land use change is one of the most powerful contributors to climate change (Andrén, Kätterer and Karlsson, 2004; Lehuger, Gabrielle and Gagnaire, 2009; Ball, 2013). It can be argued that importing grain to support British livestock is exporting some of their environmental impact (Zaks *et al.*, 2009; Henders, Persson and Kastner, 2015), especially as GHG emissions are generally attributed to the producer, not the consumer. This is despite up to a third of the product of nations suffering high deforestation being embodied in exports (Henders et al., 2015). Conversely, this would also work in the reverse if Britain was producing meat to export, but the UK produces the equivalent of its consumption in meat, milk and eggs while exporting some lower quality beef and dairy and importing some higher quality the meat (DEFRA, 2021c).

6.4.2 Nitrous oxide

A generalised linear model (GLM) for the response variable of soil N_2O in g m^{-2} y^{-1} produced no indication that any of the predictors were statistically significant drivers.

In the literature, climate, precipitation and temperature are significant drivers of difference in N_2O emissions (Skiba *et al.*, 1992). This is because elevated soil water content encourages microbial growth and soil microbial N_2O production (Barton *et al.*, 2008). Reduced water availability limits N cycling in beech forest soils and limits bioavailable N production (Dannenmann *et al.*, 2016). However, water is not likely to be the most limiting factor on N_2O production in Britain and British-aligned climates. Fig. 6.2 shows the negligible correlation between precipitation and N_2O release. Heat, drought and flooding are also influential climate factors in microbial release and consumption of bioavailable N (Rennenberg *et al.*, 2009), but these are unlikely to be deciding factors in this meta-analysis where the focus was on British climates and those comparable (temperate maritime). It may be that differences in climate, in this review with limited scope, were not detected in the model due to the deliberate elimination of studies with semi-arid or arid soils and significant differences in precipitation.

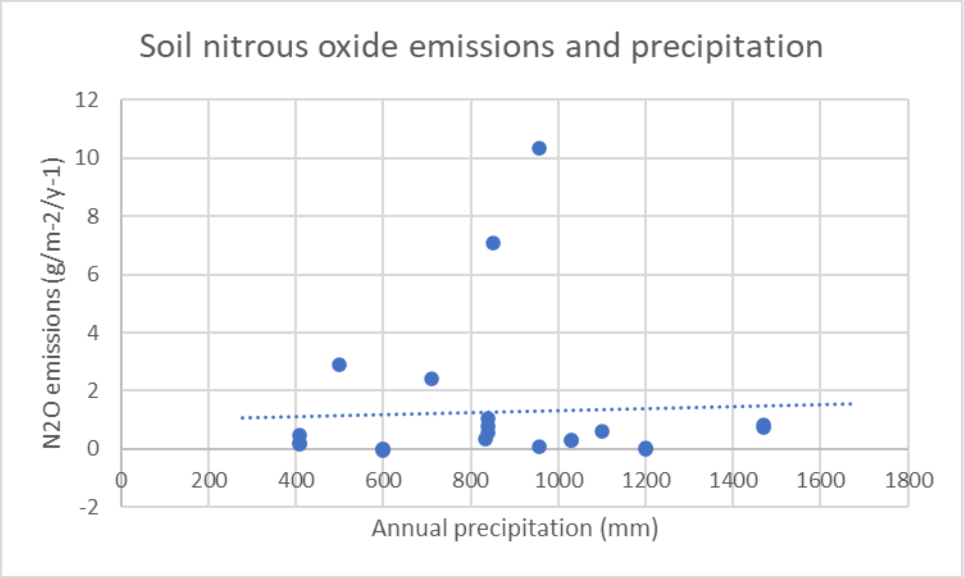


Figure 6.2 Soil N_2O emissions relative to precipitation

Grazing has also been linked to increased soil N_2O emissions, despite a lack of correlation in this research. Fig. 6.3 shows the slight negative correlation visible in the raw data between N_2O emission and livestock grazing pressure in LSU. N in excreta on the soil surface increases soil N_2O emissions, and soil management practices to support livestock such as ploughing increase soil N exposure to air (Luo *et al.*, 2010). Proposals for reducing soil N_2O emissions related to grazing livestock include limiting N fertiliser, especially when soil is wet for the reasons discussed above; feeding low-N supplements; selecting ley plant species for increased N use efficiency so lower inputs support the same biomass (Luo *et al.*, 2010). However, increases in nitrogen stocks have also been linked to grazing, although there seems to be less evidence for this effect being consistent and variation is driven more by individual farms (differences such as livestock species/breed, LSU, intensity, and existing substrate) than direct management (Contosta *et al.*, 2021). Fig. 6.3 could suggest evidence in support of this theory with a weak negative correlation, where increased LSU results in reduced N_2O emissions. This could indicate that differences between farms in the study were not enough to drive significance in the model, despite links in the literature. However, that there is evidence for both sides of the grazing argument with regards to N_2O emissions suggests existing research is not decisive and there is scope for future research here.

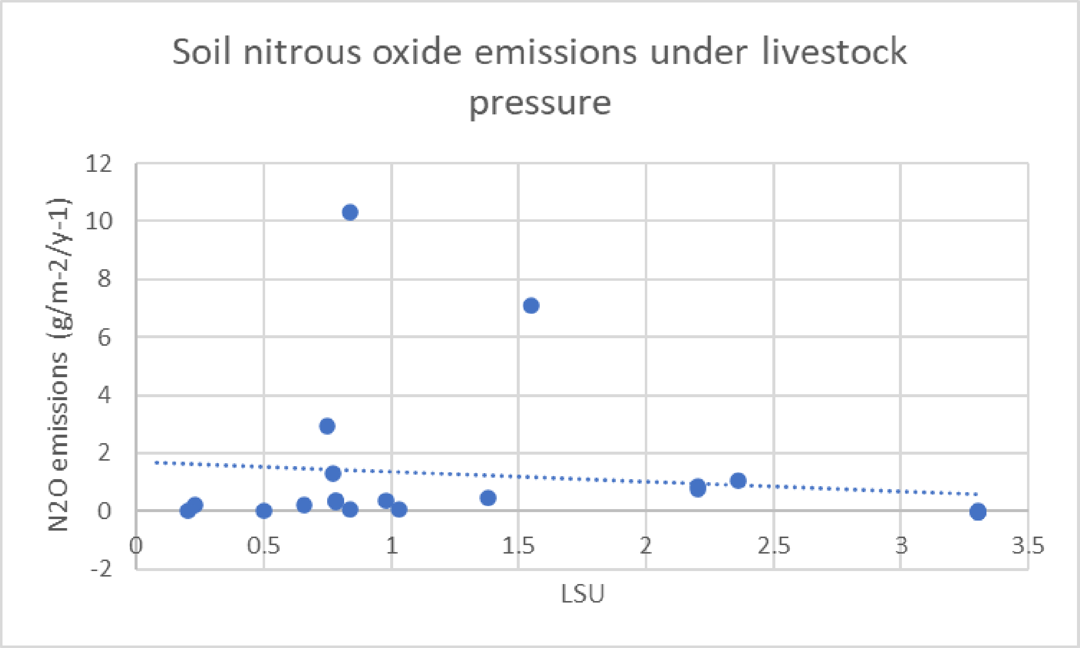


Figure 6.3 Weak negative correlation between LSU and N_2O emissions

The null hypothesis must also be accepted due to a lack of evidence for grassland type or grazing type influencing N_2O emissions. Grazing type is linked to LSU, discussed above, because unimproved pasture does not support a high density of animals for a sustained period of time, and it is inefficient to improve a field and then stock with a very low density of animals. Therefore intensity and improvement are linked, another reason a variety of studies across were difficult to find and include in the meta-analysis.

A main driver of soil N_2O is soil aeration (Skiba *et al.*, 1992), which was not a variable included in this analysis, which could explain some of the data in the model where other variables have failed to do so. Soil aeration and water-filled pore space are important, with water-filled pore space holding a strong positive relationship with N_2O emissions because of increased oxygen and water for microbial activity and respiration (Gillam, Zebarth and Burton, 2011).

C content of the soil is also an important factor in the abundance and partitioning of N losses in gas due to denitrification, with rising N_2O emission with increased C inputs (Gillam, Zebarth and Burton, 2011). Baseline soil C content was not directly measured in the studies, or included as a variable in the meta-analysis, only C flux. Omission of this variable is likely to be a further limitation on the effectiveness of the model as a predictor of soil N2O emissions and why there is a lack of significant influencing variables.

Mycorrhizal fungi and bacteria also produce N compounds including N_2O and NO, and these communities are more active in soil when N-limitation is removed as a growth control factor (Rennenberg *et al.*, 2009), which is more likely in improved soils with organic or inorganic inputs. However, N inputs are also not included in this model, which could explain some of the data. This is a further limitation of this research.

Extrapolation of the Skiba et al. (1992) data to the whole of the UK showed that agricultural land may account for 2-6% total annual NO emissions and up to 64% total UK N_2O emissions. Therefore, a full account of N_2O emissions across different grassland types, farms and production strategies is necessary for taking steps to reduce emissions, by targeting reductions to where they will make the most impact. Because of the lack of power in this GLM in terms of explaining N_2O emissions by livestock, grassland or grazing type, it is important that future research along similar lines take into account the variables discussed above to establish a clearer picture of the direct influences on soil emissions and how we can target processes and management to reduce them, if indeed livestock or grass or grazing type are not significant drivers.

6.4.3 Methane

In contrast to nitrous oxide, methane efflux is driven by multiple significant predictors.

Mediterranean climate and precipitation are very strong predictors of CH_4 emissions. Fig. 6.4 helps illustrate why the model detects Mediterranean climate, and precipitation, as such strong predictors but fails to differentiate between UK and other temperate maritime climates. CH_4 emissions are significantly reduced compared to soil in more temperate climates. Like N_2O, this is because of microbial activity. Methane is produced in anaerobic subsoil by methanogens, a group of microorganisms, and wetter soil is a greater source of methane because water prevents oxygen ingress and provides anaerobic conditions for the breakdown of organic matter and methanogenesis (Le Mer and Roger, 2001). The moisture in soils with much higher precipitation (UK and analogous climates) produces greater rates of methane production, and grasslands and cultivated soils are second only to forest soils by rate of methanotrophy (Le Mer and Roger, 2001). Alternatively, aerobic and dry soils can act as a significant CH_4 sink; around 6% the CH_4 produced annually in Australia is absorbed back into the country's arid soils (Dalal *et al.*, 2008).

The relationship between methanogens and methanotrophs (the producers of methane by anaerobic OM breakdown, and the bacteria which oxidise that methane to carbon dioxide in the top soil layers), frequently mediated by moisture related to climate conditions, also explains the relationship between CH_4 and CO_2 detected in the model.

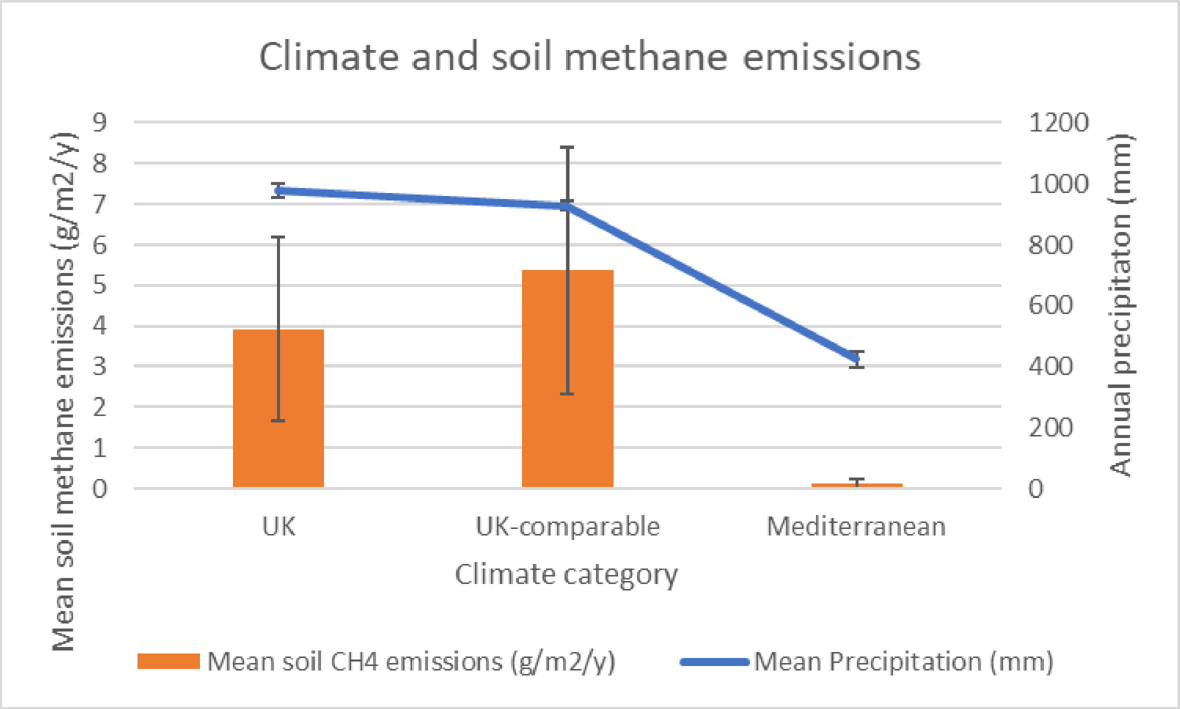


Figure 6.4 Correlation between climate and precipitation, and rates of soil CH_4 emission

Semi-natural lowland grassland, semi-improved grassland and improved grassland were also strong predictors of CH_4 emissions. The grassland category the soil supports has a significant influence on the volume of methane emitted, for many reasons. These include varying levels of OM or inorganic inputs, nitrogen inputs, mowing and grazing. In the van den Pol-Van Dasselaar et al (1997) experiment, mowing without N application achieved a net sink effect, but all other treatments involving applications or grazing acted as net sources of methane. Applying N fertiliser increases methane efflux from the soil because the consumption of CH_4 in the top layer of bioactive soil decreases, potentially because N inhibits methanotrophs (which oxidise CH_4 to CO_2) by ammonium or nitrite, or because repeated fertiliser application changes the abundance, functional group, and/or diversity of the microbial community (A. Van Den Pol-Van Dasselaar, Van Beusichem and Oenema, 1997). Methanogens compete with nitrate-reducing bacteria for hydrogen and other organic sources, and methanogens fix nitrogen which nitrate-reducing bacteria require (Prem, Reitschuler and Illmer, 2014). Managements such as mowing and fertiliser inputs are more common on semi-improved and improved sites than semi-natural sites, and this is likely to be driving the differences between grassland types, via the medium of microbiota. Fig. 6.5 illustrates the differences in mean and SE between the methane emissions of different grassland types. It is likely that semi-natural upland doesn't feature as an influence solely because of the statistical weakness of the test, as only two papers had data for methane emissions from semi-natural uplands. Grasslands have previously been considered net sinks for methane (A. Van Den Pol-Van Dasselaar, Van Beusichem and Oenema, 1997), but this meta-analysis provides no evidence that any kind of grassland is a net sink, although semi-improved grassland is by far the greatest contributor to soil CH_4 emissions.

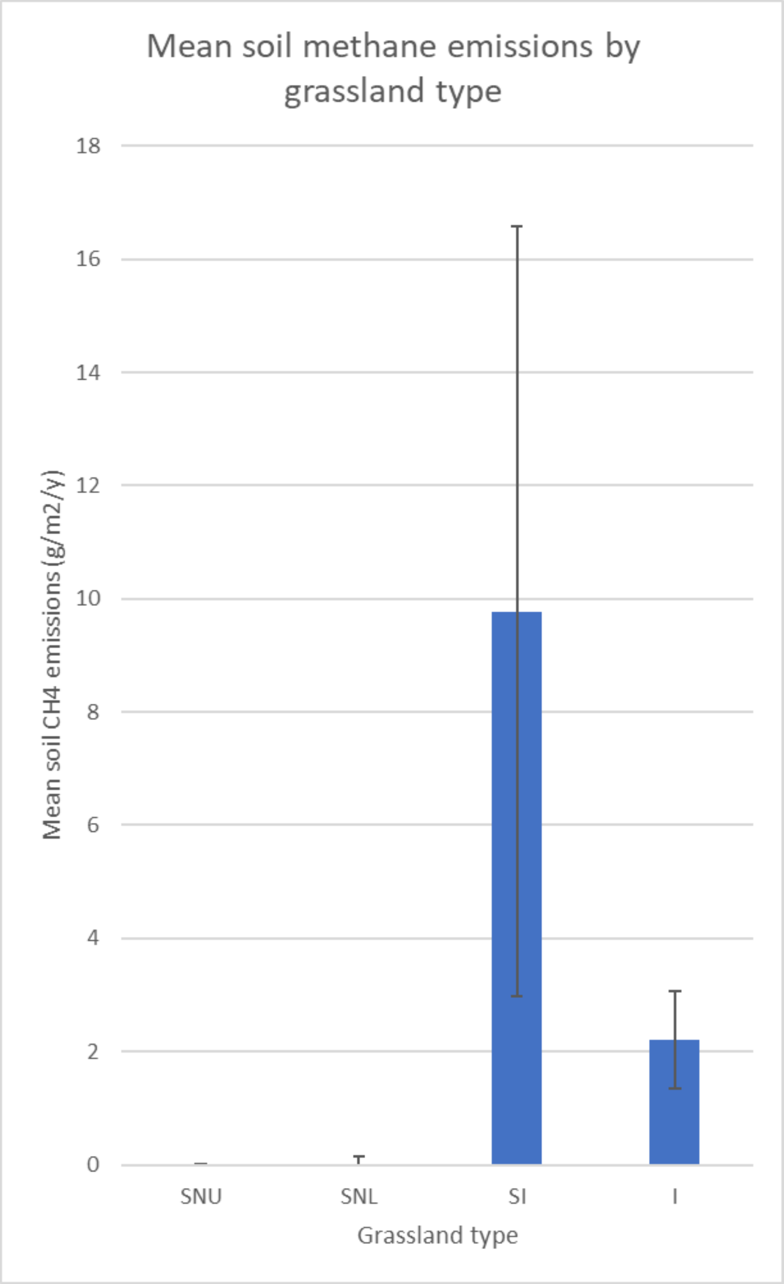


Figure 6.5. Differences in methane emissions between grassland types (SNU= Semi Natural Upland; SNL = Semi Natural Lowland; SI = Semi Improved; I = Improved)

LSU also proved a significant driver of soil CH_4 emissions. Fig. 6.6 illustrates the weak positive correlation detected in the GLM. Livestock grazing changes the soil biota, and influences soil abiotic characteristics, and soil microbiota are the main driver of soil methane emissions (Le Mer and Roger, 2001; Hiltbrunner *et al.*, 2012). Obligate anaerobic methanogen Archaea produce 70-80% atmospheric methane (Prem, Reitschuler and Illmer, 2014) Pasture soils have high potential for methane production due to organic matter inputs from fertilisers to improve grass yield and also from livestock excreta, with the potential CH_4 loss to the atmosphere increasing with grazing intensity (Prem, Reitschuler and Illmer, 2014). Livestock-driven processes are related to the N enrichment discussed above and methanogen-bacteria competition, but also influences other processes. Ammonia (common in urine) enhances plant growth, which can increase the concentration of soil C for fermentation, a process which releases CH_4 (Prem, Reitschuler and Illmer, 2014). OM in the form of manure, or even the treading in of plant biomass, can stimulate mineralisation which increases microbial activity and therefore reduces the oxygen availability in the soil which is beneficial for obligate anaerobes (Prem, Reitschuler and Illmer, 2014). Poaching also increases water cover in intermittent patches, increasing the soil area likely to be without oxygen. Given that the inputs increase as LSU increases, there are several routes by which increased livestock might influence soil methane emissions, via impacts on the microbial community.

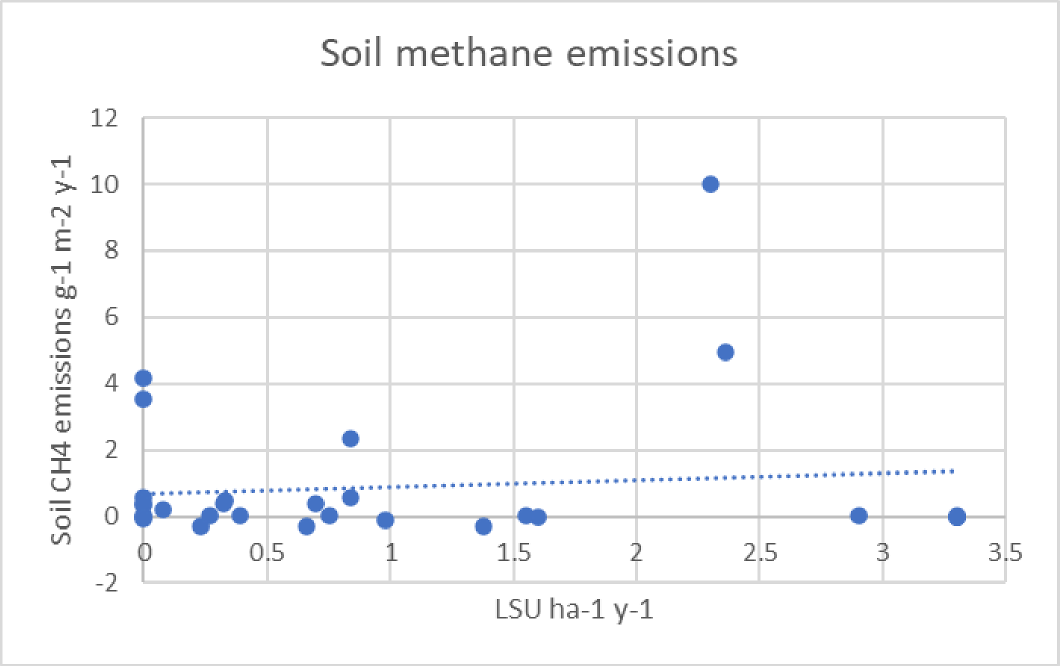


Figure 6.6 Soil methane emissions and grazing pressure

6.4.4 Carbon dioxide

It might be expected that the processes which generate soil CH_4 would also influence N_2O and CO_2, as similar conditions might support a variety of gas-producing microorganisms. Inputs of OM are known to affect CO_2 production as they do CH_4 production, but by different organisms and processes. Where OM is reduced by tillage, CO_2 flux from the soil often increases (Reicosky, Dugas and Torbert, 1997), however, organic matter inputs and grazing have also been linked to higher OC content in soil (Thomas *et al.*, 2017). This illustrates similar processes having opposite effects (but still detectable effects) on soil emissions of both CH_4 (as discussed in section 6.4.3) and CO_2. This is why there are significant overlaps in the variables which influence CH_4 and CO_2 production in soil. That N_2O production is not influenced by any of the predictor variables indicates a significant limitation in the GLMs discussed, in that microbial data was not included in the meta-analysis. Future research could engage better with this topic, especially around N_2O, as patterns were not established and significant drivers were not detected. However, microbially-mediated effects on CH_4 and N_2O are detectable in these models, through the indirect variables influenced by microbes or by grazing, which create feedback effects.

Variables influencing feedback loops, which affect soil CO_2 flux, include precipitation. Soil CO_2 emissions are up to 11.5% higher in irrigated grassland plots than dry grassland plots, indicating that CO_2 production is a water-limited process (Risch and Frank, 2007). Increased water not only allows increased microbial activity but also increased plant growth, which will increase OM deposition into the soil from rhizodeposits and root tissues, which gives microbiota fuel to break down for these reactions (Hafner and Kuzyakov, 2016). Seasonal water-linked changes in CO_2 flux have also been observed in grasslands of native and non-native species ranches, where GHG emissions were mediated by the rate of nutrient mineralisation, a process influenced by water and temperature (Peterson, Starks and Steiner, 2021). Seasonal fluctuations in N, water and soil temperature drive increased CO_2 emissions in spring to summer, the growth period, by way of increased microbial respiration (Peterson, Starks and Steiner, 2021). Additionally, water influences abiotic drivers of soil processes such as carrying nitrates and nitrates which cause pH changes, and changing BD, which affects the permeability of soil to root biomass which is a main source of belowground C to fuel microbial processes (Peterson, Starks and Steiner, 2021). However, the results of the meta-analysis provide evidence potentially for the opposite effect: there is a negative relationship between water and CO_2 emissions, visualised in the basic graph below (Fig. 6.7). This means that microbial respiration, limited by water, is not the deciding factor on the CO_2 emissions in this meta-analysis. There is evidence on both sides, where increased precipitation has been shown to both increase CO_2 loss and increase CO_2 sequestration, which means the drivers of the effect are far from well established. The deciding factor on CO_2 sequestration or emission is likely to be a balance between belowground biomass production or microbial respiration. Belowground biomass accumulation, mainly by plants, encourages sequestration in the soil by retaining tissues which are slowly degraded into less labile C pools (as discussed in Chapters 3 and 4), reliant on factors including the speed of mineralisation (Alcántara *et al.*, 2016), parent material (Bronick and Lal, 2005), or the ligneous proportion of the input (Rovira and Vallejo, 2002). Water is an influencing factor on soil C sequestration by plants in areas where plant growth is not water-limited due to adequate aboveground supply (Risch and Frank, 2007). In contrast, microbial respiration may be likelier to tip the balance towards soil C loss if an excess of water influences the rate of mineralisation, and/or seasonal fluctuation influences abiotic factors which drive microbial respiration rate (Elisabeth B Ward *et al.*, 2021). Fig. 6.7 indicates that higher precipitation is linked to carbon sequestration (indicated by minus figures) rather than release in this meta-analysis, supporting the theory that temperate grazed pastures sequester more carbon with increasing precipitation. This is an important finding given that the sequestration effect takes place at the extreme end of the precipitation scale in this study. Degraded soil in arid agroecosystems has been subject to increased precipitation to improve SOC stocks (Batjes, 2001) but this has not been evidenced well in temperate soils. Increased SOM in soil, from which any CO_2 efflux is microbially derived, has been linked to better water holding capacity (Conant and Paustian, 2002) but not necessarily precipitation. High precipitation does usually support increased belowground biomass, which had a consistent positive effect on SOC storage in grasslands (Chen *et al.*, 2018). While this meta-analysis does appear to support the conclusion that more precipitation is better for increasing SOC stocks, the evidence in the wider literature is far from conclusive, and may be heavily dependent on other factors such as land use and climate (Dignac *et al.*, 2017; Raposo *et al.*, 2020).

Precipitation is a strong predictor of CO_2 and CH_4, linked by the interaction of methanogens and methanotrophs and their respective anaerobic and aerobic water-linked processes. This climatic feature also explains why CH_4 is such a strong predictor of CO_2 (and vice-versa).

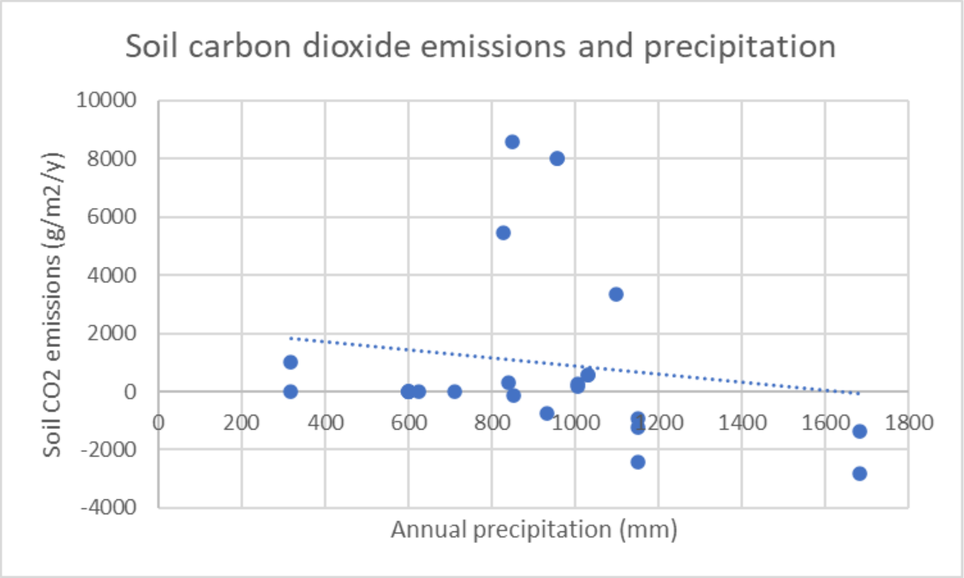


Figure 6.7 Negative relationship between soil CO_2 emissions and precipitation

Precipitation is also linked to climate and temperature, as Mediterranean climates tend to be drier and warmer (although winter data collection in one of the papers used in this meta-analysis skewed the temperature of Mediterranean-classed studies lower than average, generally temperatures in areas classed as Mediterranean climates are higher than maritime temperate areas). Experiments which account for seasonal flux find that warmer temperatures tend to drive microbial activity, as they are linked to plant biomass and microbial biomass production, the bioavailability of C and N, and increased rates of mineralisation for OM (Peterson, Starks and Steiner, 2021). Higher temperatures have been linked to higher soil CO_2 emissions in semi-arid soils (Bell, Eckard and Cullen, 2012), although results are very variable between different soils and climates and how they are put into production and their modelled emissions under warming scenarios (Del Grosso *et al.*, 2005; Bell, Eckard and Cullen, 2012). However, it is generally accepted that warming of temperate soils increases the rate of SOM decomposition, adding more C to the labile C pool accessible to microbiota and thereby increasing the volume of CO_2 released to the atmosphere from temperate soils (Jenkinson, Adams and Wild, 1991). That both UK-adjacent and Mediterranean climates predict changes in CO_2 efflux indicate water- and temperature-mediated processes are responsible for driving microbial CO_2 production.

Sheep as the grazing livestock, and the presence of both sheep and cattle, were detected as significant drivers of differences in CO_2 emissions in the GLM. However, there may be more to this than there initially seems, because there were much fewer sheep-based studies (10) than cattle-based (35), and even fewer with both sheep and cattle (four), see Table 6.5. Also, because of a dearth of temperate-maritime or Mediterranean studies on the effect of sheep grazing, this small pool of sheep-based studies in the meta-analysis was more likely to come from a British study than other climates (including precipitation levels and temperatures), so there may be linked factors at play which are the result of bias in the pool of available studies rather than direct effects. However, having acknowledged this, there are differences in the way sheep and cattle graze which may influence CO_2 production. For example, sheep prefer grazing tender new growth and cattle prefer tough or fibrous tissues, and sheep graze lower in the sward and cut growth close to the soil, whereas cattle leave grass as a longer sward, hence their benefit in low numbers for encouraging biodiversity in grasslands as they don’t cut off the plant too low but discourage grasses outcompeting herbs (Wrage *et al.*, 2011; Török, Kapocsi and Deák, 2012). The presence of sheep in a study, therefore, means grasses are likely to have been cut off shorter in the sward and herbs grazed preferentially, whereas both sheep and cattle mean a short sward, with herbs, grasses and more ligneous plants grazed at varying heights. Generally, grazing aboveground biomass encourages compensatory root growth, which contributes to SOC increase (Pucheta *et al.*, 2004; Harvey *et al.*, 2019). Sheep grazing may have a different effect on soil CO_2 emissions to cattle grazing, because sheep grazing does not produce an increase in available soil C and N unless their grazing is combined with tillage (which is the main driver) (Lazcano *et al.*, 2022). In contrast, cattle grazing may remove more C from the soil as they are larger herbivores and less efficient grazers, losing more of the C they consume from plant biomass in excreta and by respiration, and in dairy products removed from the nutrient cycle, as these are less in demand from sheep (Garnett *et al.*, 2017). There is evidence that sheep grazing does reduce SOC stocks (Golluscio *et al.*, 2009), but it is difficult to find further evidence in the literature comparing sheep to the same LSU of cattle. However, these effects are far from established in the literature, as the majority of research on animal grazing focuses on enteric emissions and not the effect of grazing on soil emissions. This has been a common theme throughout the meta-analysis and is considered a limitation of this research due to the lack of available data. Fig. 6.8 below shows that, without any further detail in terms of grassland type or grazing intensity, that sheep grazing does produce a significantly higher soil CO_2 output than cattle grazing, although this should be viewed while acknowledging the potential biases in terms of the limited data.

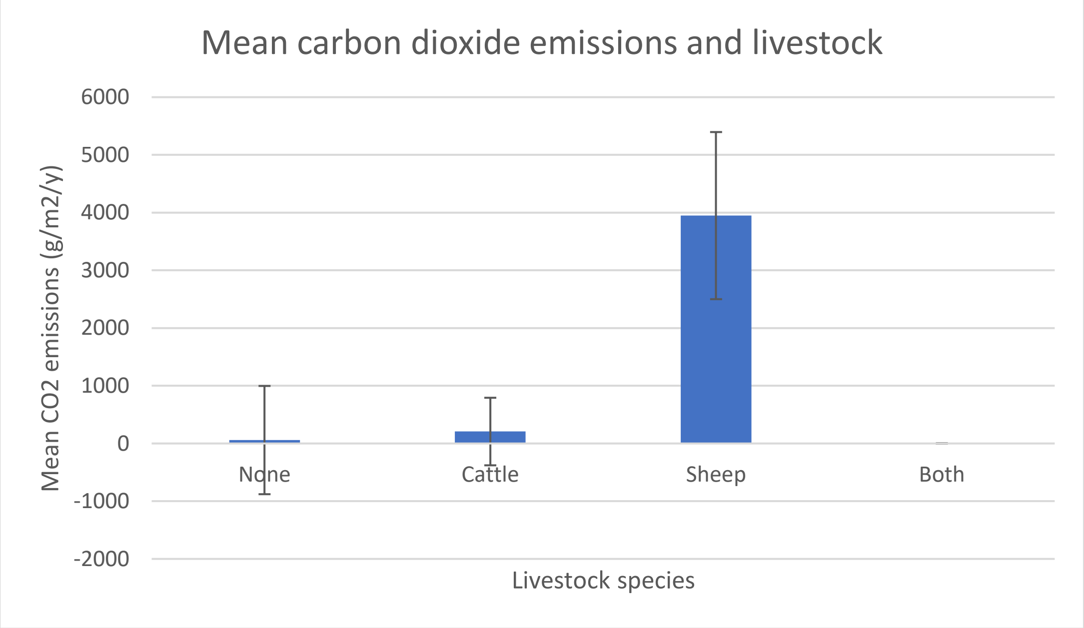


Figure 6.8. Mean soil CO_2 emission from sheep-grazed pasture is significantly higher than from cattle-grazed

Grassland type, grazing intensity and LSU were not significant predictors of CO_2 emissions. This is despite evidence that open grazed rangelands produce significantly lower CO_2 efflux than pasture enclosures, where vegetation was manipulated animals were more concentrated (Oduor *et al.*, 2018). Likewise, there is no evidence that grazing intensity and grassland type (generally linked by monoculture and increased inputs for efficiency) influenced soil CO_2 emissions in this study, despite evidence in the literature that monoculture and non-native grasses produced higher CO_2 emissions from the soil than native and diverse pastures (Peterson, Starks and Steiner, 2021). Concentrating animals in smaller areas of pasture has more in common with semi-improved grasslands than semi-natural grasslands, and the higher the LSU, the higher the CO_2 efflux in rangelands (Oduor *et al.*, 2018). Increased grazing pressure has been linked to increasing soil CO2 emissions (Schulz *et al.*, 2016; Mei *et al.*, 2018; Oduor *et al.*, 2018) but despite this, no correlation is visible between LSU and CO2 emissions in Fig. 6.9 below, a basic illustration of the lack of trend. What the model and trend line do not detect, however, is that a C sequestration effect takes place only at very low values of LSU (Fig. 6.9). It is important to consider that there could be mediating factors in the research used to build this meta-analysis, such as a relative climatic stability across the study, with the number of papers in UK or UK-adjacent climates more than double the number of papers in semi-arid climates. This introduces a source of bias where a lack of relationship may have been undetectable due to a lack of evidence rather than a lack of true difference. Additionally, the soil CO_2 data analysed in the meta-analysis had an extraordinary variability, as illustrated in Fig. 6.9 below, but none of the datapoints in the figure are outliers. This may also be a partial explanation of why patterns are so difficult to detect in the model.

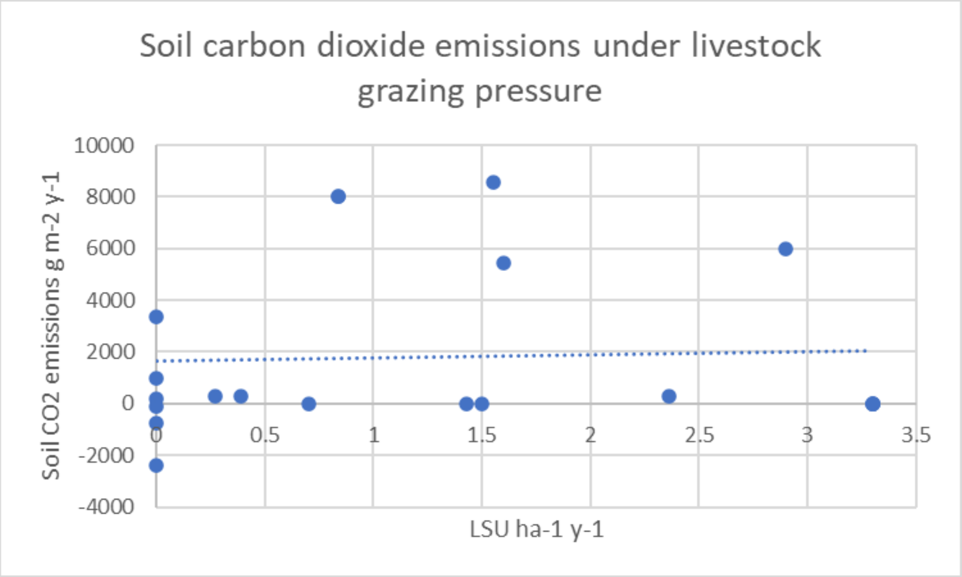


Figure 6.9 Soil CO_2 emissions under livestock grazing pressure

6.4.5 Comparisons over grassland and grazing systems

It is not helpful to visualise N_2O and CH_4 emissions on the same axes as CO_2 emissions, as CO_2 values are much higher and render differences in the other gases difficult to discern. Therefore Fig. 6.10 below is arranged only with N_2O and CH_4 as comparisons compared across all grassland-grazing systems.

It appears from Fig. 6.10 that improved grasslands, if they have been established for several years, reach a more constant state of C input and output, as discussed in Chapter 1, more readily than grasslands in other stages of improvement or intensity. The SEs are comparatively closer about the mean than N_2O in semi-natural upland or CH_4 from semi-improved grassland states, suggesting more stability in soil processes generating C-containing compounds including CH_4, and N-containing compounds including N_2O. However, this relative constancy is in the wrong direction, with consistent C and N efflux from the soil. Semi-natural grasslands are much more stable and unproductive than both improved and semi-improved grasslands in terms of CH_4 emissions, providing strong evidence that semi-natural (and by definition, low LSU) grasslands are better for reducing CH_4 emissions than improved. CH4 emissions are negligible in semi-natural grasslands compared to improved and semi-improved. Semi-improved is by far the most CH_4 productive category, which is perhaps down to the wide variety of managements which can occur under the loosely defined ‘semi-improved’, but these generally involve all or a proportion of non-native species; varying levels of OM or inorganic inputs; N inputs; and mowing and/or grazing. OM or artificial inputs and grazing drive net CH_4 emissions by inhibiting methanotrophs or changing the microbial community structure (Van Den Pol-Van Dasselaar et al., 1997). Ammonia from excreta enhancing plant biomass accumulation increases soil C, then subject to fermentation, releasing CH_4 (Prem, Reitschuler and Illmer, 2014). OM in the form of manure or treading in stubble stimulates mineralisation, increasing microbial activity and reducing oxygen availability in the soil for methane-producing obligate anaerobes (Prem, Reitschuler and Illmer, 2014). In addition, statistical weakness due to fewer semi-natural experiments is likely to bias the outcome.

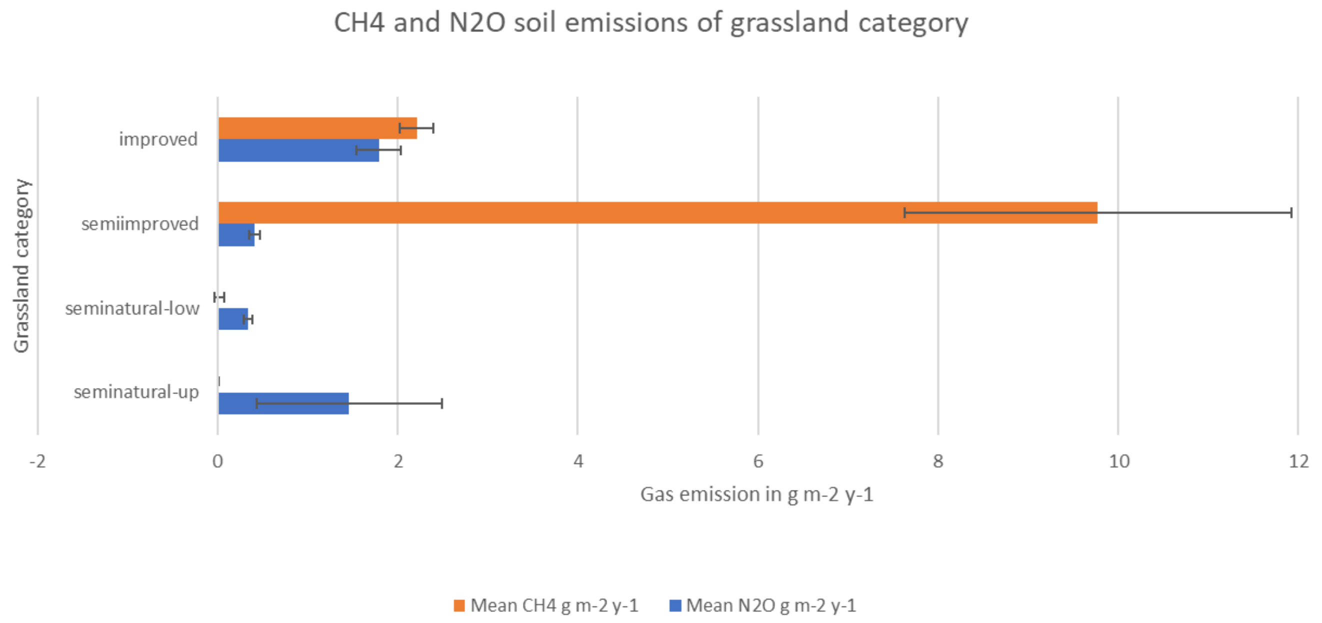


Figure 6.10 A comparison of CH_4 and N_2O emissions across grassland categories

A further illustration of the influential variables of grassland and grazing type, identified in the GLMs, is below in Fig. 6.11. Here, grassland is divided also into grazing type, illustrating greater distinction between the classes than Fig. 6.10. We can observe that much of the difference in the semi-improved emissions shown above is driven by intensive and cattle-grazed pastures. Sheep grazing, detected as a powerful predictor in the GLMs, is driven mainly by grazing improved grasslands, and a greater proportion of that is from extensive grazing. This is expected because grazing extensively on an improved grassland is less efficient in terms of food production for the amount of inputs the land receives, and much of the land may be subject to leaching or N run-off which will increase GHG emissions (Muhammed *et al.*, 2018). Extensive cattle-grazed semi-natural lowland is the only management strategy consistently producing a net soil uptake of C, likely due to the low LSU allowing plant biomass to recover, increasing plant diversity by reducing grass dominance, and through low or no inputs (Mazzetto *et al.*, 2015; Wiesmeier *et al.*, 2016; Yang *et al.*, 2019).

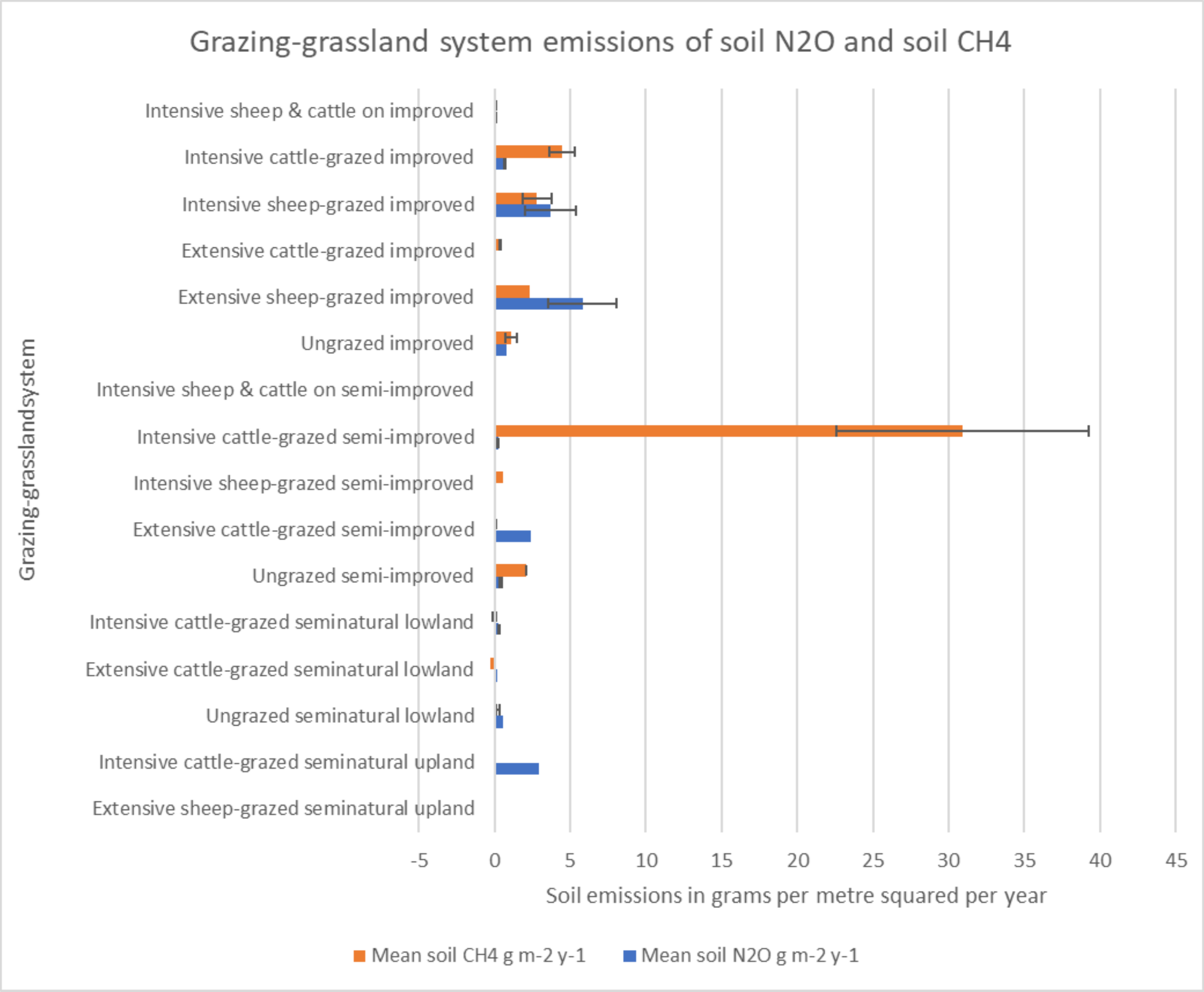


Figure 6.11 Grazing-grassland systems by CH_4 and N_2O output

6.4.6 Common variables with a soil sequestration effect

N_2O

Only three experiments out of 59 had a negative N_2O value, which itself may indicate an undetected bias in the study, or a significant variable which has not been included in the meta-analysis, particularly as the three experiments were from the same paper (Manono, 2016).

The LSU was particularly high, at triple the grazing pressure designated as ‘intense’ on improved grassland, which extrapolates to supporting an LSU of more than double the UK’s current grazing population. This is surprising where there is a net uptake of N_2O into the soil because of the influencing factors on N_2O flux including water availability (Dannenmann *et al.*, 2016) and reduced N inputs (Luo *et al.*, 2010) and grazing (Contosta *et al.*, 2021). A likely driver of difference in the case of the Manono (2016) experiments is grazing, as this was the only paper to utilise simultaneous sheep and cattle grazing. Increases in nitrogen stocks have been linked to grazing, although this effect is driven more by individual than direct management, and shows variability between farms (Contosta *et al.*, 2021).

Reduced water availability limits N cycling in forest soils by limiting bioavailable N (Dannenmann *et al.*, 2016), and Manono (2016) had a lower annual precipitation than temperate maritime studies; however, there are other studies from drier soils which do not reflect this N_2O sequestration effect (Beauchemin *et al.*, 2010, 2011; McGinn *et al.*, 2014; Stewart *et al.*, 2014).

The sequestration was very small (-0.024 ± 0.007 g m^{-2} y^{-1}) but nonetheless, an important result given the GWP of N_2O at 298 times that of CO_2. However, N_2O cannot be considered in isolation and this experiment did not occur in the selection of papers showing net sequestration of CO_2. The two experiments on intensive grassland did, however, appear in the papers showing CH_4 sequestration. This indicates that one or more of the approaches taken in the Manono (2016) experiments, particularly on improved grassland, are integrating positive management strategies for both C and N sequestration.

Reflecting on the potential for UK grassland to support the livestock grazing pressure suggested by Manono (2016), this is extremely unlikely to work in practice in the UK for several reasons. Firstly, the diversity of grassland and the LSU supported is vastly different across even ‘intensive’ and ‘extensive’, or ‘improved’ and ‘semi-improved’, with much variety within these definitions. N_2O sequestration is, as discussed above, extremely farm-specific (Contosta *et al.*, 2021) and farm specificity includes legacy effects from previous land use or treatment (Reid, 2019); which crops are in rotation (Smith, Gross and Robertson, 2008); length of time of ley establishment (Jarvis *et al.*, 2017); the species and diversity in the ley (Jordon *et al.*, 2022); the form and intensity of grazing (Schönbach *et al.*, 2012); even the parent material of the soil (Erktan, Or and Scheu, 2020).

Secondly, Manono (2016) reflects many environmental conditions which will not be replicated in the UK. For example, the rainfall was around half that expected in the UK, and soil moisture is a significant driver of N_2O emissions as discussed above. Additionally, just over half the UK’s grassland is improved (Nafilyan, 2015), whereas Manono (2016) relies on all of it being improved, and all of it being intensively grazed at a very high LSU. These intensive and improved conditions are unsustainable for many reasons, even if it were true that the soil would become a net N_2O sink if they were extended across all British grassland. The enteric emissions of such a growth in livestock population would far outweigh any benefit in soil sequestration (Cunha *et al.*, 2016), as would the additional production and transport of feed and fertiliser (Brock *et al.*, 2012; Henders, Persson and Kastner, 2015). Reshaping the landscape also ignores the other ecosystem services supplied on these grasslands including water filtration, nutrient capture, flood defence, and habitat provision for other wildlife (Johnston, 2014). Even if the principle were to work in reverse, and livestock populations maintained at current levels but at higher LSU on less land area, this still leaves no room for common managements which are established for improving soil health and maintaining biodiversity, such as conservation grazing (The Scottish Agricultural College, 2007), mob grazing, or maintaining occasionally grazed wildflower meadows (Eriksson, Cousins and Bruun, 2002).

Assessing the variables built in to the experiments with net N_2O gain was intended to illustrate the potential for sequestration in the UK, but because of the differences to UK habitat, climate, and agricultural systems, this is not an appropriate management strategy. LSU was also not indicated as a significant predictor of soil N_2O emissions, unlike CH_4 and CO_2, which means that livestock reductions (or increases) are probably not the most reliable point from which to make assumptions on soil N_2O emissions. There are many other considerations which mean LSU should be much lower and a small net N_2O uptake is extremely costly in other areas related to GHG emissions and climate change mitigation. The extremely limited number of experiments achieving a net sequestration effect of N_2O raises further questions about the robustness of Manono (2016) when this was the only paper to achieve N_2O sequestration, and did so across three experiments, compared to 56 other experiments which failed to reach a net influx of N_2O into the soil. This is not a reliable basis for a large scale policy change and instead requires much more research to investigate additional variables which could produce such a result, and how these might transfer into a temperate maritime climate in the UK, and across the varied grassland system in British agriculture.

CH_4

Only 11 experiments out of 59 had a negative CH_4 value. This is a slightly more significant number than for N_2O, from which conclusions might potentially be drawn. However, it indicates that across three climates, a great range of LSU values, different farming systems, models and experiments, less than 20% achieved a net gain of CH_4 into the soil rather than a loss.

Broad scale patterns to discuss include that 72.72% were in temperate maritime climates, so this may be a driving factor. While increased precipitation may increase CH_4 emissions from the soil due to obligate anaerobes generating the majority of soil CH4 (Prem, Reitschuler and Illmer, 2014), not all soil is submerged in high precipitation areas and an aerated soil with sufficient moisture to support bacteria will also have a healthy population of methanotrophs, which consume the products of methanogenesis, producing CO_2 (Le Mer and Roger, 2001) which has a much lower GWP.

The mean LSU of 1.64±0.55 is fairly average for intensive improved grassland in the UK although there are no nationwide statistics on LSU. However, extrapolating this LSU suggested by experiments which achieve CH_4 sequestration produces a potential 9.2 million LSU. The UK is currently supporting a higher population of livestock than this, at around 12.9 million LSU, although these are not supported solely on grass but by supplementary feeding. Given that only 56.33% the 5.6 million hectares of UK grassland is improved, reducing LSU to 1 on the rest of the grassland (assumed semi-improved, which as we know is also an overestimate because of semi-natural grasslands) produces a potential LSU of 7.7 million, significantly less than our current population, which could be sustainably supported on existing grassland whilst achieving net CH_4 sequestration in the soil.

However, this does not take enteric emissions into account. Enteric emissions from the four studies which had both enteric emissions data and a net soil CH_4 sequestration effect show that enteric emissions far outstrip the CH_4 absorbed into the soil. Mean enteric emissions in g kg liveweight^{-1} d^{-1} extrapolated up to the average liveweight of cattle at the suggested CH_4-positive LSU of 1.64 produce an annual mean CH_4 enteric emission of 46731.98±35423.04 g over each hectare. This is in comparison to a mean annual net uptake by the soil in all 11 experiments of -0.13±0.04. This is still producing a heavy net loss of CH_4 to the atmosphere by farming livestock, despite grazing on intensive improved grasslands achieving slight soil sequestration.

Additionally, no-grazing experiments were again overrepresented in the proportion of studies remaining as examples of CH_4 sequestration. Manono (2016), with the potential flaws discussed above, again contributed two experiments to the 11 which had negative CH_4 values.

Reflecting on the potential for UK grassland to support the livestock grazing pressure suggested by these 11 experiments, the main limitations would be in the established businesses, subsidy systems and legacies of farmers and farmland which has been under significant livestock production for generations. Potential for change towards a lower livestock population is on the horizon with the roll-out of post-Brexit changes to farm funding (ELMS), which may well incentivise some land use change from less productive (low input) grasslands to more nature-based solutions. In terms of soil CH_4 sequestration potential, the UK is currently overshooting its CH4 soil emissions even by this limited estimate across 11 experiments. The evidence here suggests that turning soil into an effective CH_4 sink would require a lower LSU than that currently supported across many farms, especially in the lowlands, and that enteric emissions would greatly outweigh any climate-related benefit of CH4 sequestration unless the livestock population was hugely curtailed.

$\underline{CO_2}$

Following the same process as above, retaining only experiments which resulted in a net gain of soil CO_2 produced eight results, of which seven have a similar climate to the UK and one has a Mediterranean climate.

Mean annual precipitation over all experiments is 1150.5 mm and mean annual temperature 7.8°C. Because of the climatic and moisture-related link to CH4 production by anaerobes, low CO_2 production may be linked to a moisture content enough to support an active bacterial community but not enough to create a significant anaerobic zone (Prem, Reitschuler and Illmer, 2014). Instead, net uptake of both GHGs into the soil outstrips methanogenesis or methanotrophs producing CO_2 by breaking down methane.

37.5% the studies showing carbon sequestration had no grazing at all, despite making up only 15.25% the total number of studies including net C loss. This indicates that generally, less or no grazing is a more effective route to soil C sequestration than grazing, but that grazing is very overrepresented in this selection of papers. This should be explored further in future research, as many studies are focused on grazing for livestock production given the scale of land use directed towards it. If further evidence could be provided that other ecosystem services, namely CO2 sequestration, could be provided above food production which are equally valuable (in terms of climate regulation), land without grazing might become a more accessible study topic, and a more widespread decision by farmers and landholders.

50.00% the sites were cattle-grazed and 12.50% grazed sheep. However, only one study had an LSU value available. This was 1.43 and it is for the study in the Mediterranean climate, cattle-grazed on intensive improved grassland. Therefore this is not a representative value of LSU across the studies, due to climatic differences which, as discussed above, can be very influential on soil GHG production.

Seven out of eight sites were on improved grassland and one on semi-improved, which again points to bias in the meta-analysis and in the body of literature available, as there is a shortage of data on less improved and less intensively grazed sites. This is not necessarily a reliable indicator of CH4 sequestration in itself due to the bias in the number of improved grassland sites.

Assuming an LSU value of 1.43 across all grassland in the UK, this would support 8.0 million LSU (equivalent to 8.0 million cattle or 50.0 million lowland ewes). However, only 56.33% the 5.6 million hectares of UK grassland is improved, producing a potential value of 2.0 million LSU at 1 LSU ha^{-1} on semi-improved grassland and 4.5 million LSU at 1.43 LSU ha^{-1} on improved grassland. This totals 6.5 million LSU. This is significantly lower than the current LSU supported wholly or partially on British grasslands, which reaches 12.92 million. This indicates that in order to achieve net CO^2 sequestration across British soils on the existing area of grassland, livestock populations must be much lower. This is without considering enteric emissions which have a much greater CO^2-eq value than any soil sequestration value.

6.5 Conclusion

6.5.1 Land use context

Improved and semi-improved pastures account for 39% the UK land area. The scale of grassland cover in the UK, including on high-C soils as the UK hosts over a million hectares of vulnerable peat, means that any small fluctuations in soil emissions with land use observed on one farm or one study will be proportional when extrapolated to a UK-wide scale.

The UK currently supports an approximate LSU of 2.31 ha^{-1}, but not all of this is supported entirely on grassland. Many livestock are fed supplementarily with imported feed, which means emissions associated with land use change, the production and application of fertilisers, and transport costs are exported.

Most grassland and grazing types have a net C and N loss to the atmosphere, even when enteric emissions are excluded and solely soil emissions under grazing treatments are analysed. If all British livestock were supported on British soils, a much lower livestock population would be required to bring soil back to a net sink of emissions, rather than a net emitter of GHGs. There is evidence that Britain does hold more livestock than is sustainable for maintaining soil organic C levels, supporting the initial hypothesis.

6.5.2 Modelling N_2O, CH_4 and CO_2

Modelling soil N_2O flux did not produce decisive evidence for any drivers in the selection of variables included. Grassland or grazing type, climatic factors and LSU were not influential on the total N_2O flux, despite evidence in the literature to the contrary. However, this meta-analysis does not provide further support. It is possible that other variables including soil aeration, carbon content, and microbiota are the main drivers of differences in N_2O emissions, none of which were included in this analysis. It is important to encourage further research in these areas given the large proportion (up to 64%) total UK N_2O emissions originate from agriculture.

Methane, however, responded to many of the predictor variables. Climate and precipitation are strong drivers of microbial methanogenesis because of their influence on soil aeration and microbial activity. This is also linked to CO_2 production of methanotrophs. Grassland type was also a strong predictor of soil CH4 production, because of variation in input levels affecting microbiota, and LSU is a predictor because of related processes via excreta and OM introduction to the soil. Semi-natural upland did not feature as a strong predictor, alone of the grassland types, but this may be due to a lack of data. The meta-analysis provided no evidence that a grassland type could consistently sequester CH_4, although semi-improved grasslands emitted the largest volume of CH_4.

CO_2 is closely related to CH_4 because of the symbioses between methanogens and methanotrophs, and therefore similar drivers were detected in the model. However, in this meta-analysis, a negative relationship between water and CO_2 production was identified, suggesting water-limited microbial respiration is not a primary driving force behind CO_2 loss. Climate was a consistent driver, indicating that water- and temperature-mediated processes influence microbial CO_2 production. The potential for sheep to cause a significant difference in CO_2 production could be due to grazing differences, or it could be because of the bias towards cattle-oriented studies in the literature included in the meta-analysis. However, because of the modelling result, the second hypothesis is rejected: sheep are not a more climate-sensitive option than cattle when it comes to impacts on soil carbon. Grassland type, grazing type and LSU were not significant drivers of CO_2 emissions, despite links in the literature, which may be because of a focus on a climate niche, or an overabundance of intensive and improved grassland grazing systems biasing results.

Despite a lack of significance in the model, visualising the data indicates that intensive cattle-grazed semi-improved grassland is by far the largest contributor to soil CH_4 emissions. All improved grasslands produce relatively high CH_4 and N_2O emissions compared to semi-natural grasslands (excluding sheep and cattle grazing, which was three experiments from one paper, and no other studies utilised simultaneous grazing so this has particularly low accuracy). Intensive approaches appear less sensitive to increasing LSU than semi-improved grasslands, supporting the fourth hypothesis, but they support higher fluxes than semi-natural grasslands. However, semi-natural grasslands are not an unbiased comparison given a lack of data. Only cattle-grazed semi-natural lowland achieved mean net CH_4 sequestration by category. This indicates that patterns may arise if a larger dataset could be analysed, but that there is a shortage of research among grasslands and grazing systems which are not intensive or improved. The hypothesis regarding the vulnerability of upland pastures to changing LSU has not been satisfactorily answered due to a lack of data, meaning a range of LSU values were not tested in upland pastures and further research in this area is recommended.

6.5.3 Soil sequestration effects

Removing variables which lost C and N left very few studies. Only three experiments out of 59 achieved net soil N_2O sequestration; 11 out of 59 achieved net soil CH_4 sequestration; and eight out of 59 achieved net soil CO_2 sequestration. This indicates that it may require specific conditions and may be difficult to achieve *in situ*.

Exploring the potential LSU values indicated by the experiments which achieved sequestration broadly indicated a much lower livestock population is necessary to achieve a net soil sequestration effect. N_2O was the least reliable and least applicable result, given its high LSU in a Mediterranean climate, and that three experiments derived from the same study produced a similar result that no other study achieved suggests that either conditions were extremely farm-specific and difficult to replicate, or that the experiment may be flawed. LSU was also not indicated as a significant predictor of soil N2O emissions in the models, unlike for CO_2 and CH_4.

Methane sequestration was influenced by LSU, and the studies indicated a lower livestock population than the UK currently supports would be necessary to bring soil back into a state of sink rather than source. Climate was indicated in the model as a driving factor of emissions, and temperate climates made up the majority of studies which achieved net sequestration. Incorporating exported emissions from fertiliser and feed, and the enteric emissions generated by the livestock, pushes the experiments which achieved net soil sequestration to large generators of GHGs. While an LSU of 1.64 may support soil sequestration, it does not support a net benefit to emissions reductions targets. Experiments without grazing were overrepresented in the group achieving net CH4 sequestration, indicating that more research should be investigating the effects of fallowing and reducing utilised agricultural area to provide ecosystem services other than cheap production of food items which could be viewed as luxury. Potential for change in UK policy is coming in the form of ELMS which may incentivise reduced meat production for rewilding and ecosystem service provision.

Carbon dioxide, closely related to methane production, was also lower in high-precipitation and low-temperature climates, which may be limiting methanogenesis or supporting methanotrophs. Studies excluding grazing were again overrepresented, making up 37.5% the experiments achieving net CO_2 sequestration but only 12.5% the experiments in the meta-analysis. This indicates an opportunity to explore grazing exclusion further as a means for soil CO_2 sequestration. There is also an overrepresentation of improved grassland studies, which is likely to be due to bias in the dataset rather than improved grassland being a significantly better C sink than less-grazed pastures. As only one sequestering experiment provided an LSU value, this is unlikely to be representative of all studies which achieved this; however, LSU is linked to CO_2 production and using this single value produces a result indicating a much lower livestock population could be supported if existing grassland area remained constant but the goal was to achieve net CO_2 sequestration.

6.5.4 Management implications

Because of the limitations of the meta-analysis (see below), it is difficult to make definitive recommendations for managements aimed at increasing soil sequestration of C and N from the evidence. With no significant drivers of N_2O and the much-reduced emissions from semi-natural grasslands potentially due to sampling bias, suggestions for reducing CH_4 and CO_2 from this research may be more reliable.

Broad patterns indicate that reduced inputs in the form of surplus (likely inorganic) fertilisers inhibit methanogenesis, and by altering the abundance or functional groups in the microbial community, this is a management approach which should limit methane and carbon dioxide efflux from the soil. Maintaining soil moisture at a level which supports bacterial growth but does not flood the soil to the point of creating anaerobic conditions is also likely to have a positive effect on emissions reductions. Reducing LSU, because grazing pressure influences soil OM content and the partitioning of plant resources into belowground biomass, may also reduce soil C and N loss. Sheep, particularly, seem to drive increased CO_2 emissions and should be avoided where possible. Carbon dioxide emission is also related to water and inorganic or organic inputs, and managing inputs to encourage biomass production while not enough to encourage CO_2 production is likely a difficult balance. This evidence also suggests that a substantial driver of emissions is the state of grassland being ‘semi-improved’, which could relate to a high input but lower LSU, reducing efficiency and potentially leaving areas open to run-off, increasing emissions.

6.5.5 Limitations

The major limitations of the meta-analysis are biases towards improved, and to a lesser extent, semi-improved grasslands in the literature. A greater number of studies focus on improved grassland given its prevalence across the world, and because of its important in supporting meat production. However, this is a limitation not only of this meta-analysis, but on the pool of knowledge available on agricultural land use. A narrow focus on the highest production systems does not give a balance across many different types of land use and grazing system which are not only in use, but which provide many other ecosystem services.

There is therefore a significant sample bias against semi-natural systems, which are underrepresented in the literature, and towards more intense grazing systems due to higher production rates.

Further limitations of the meta-analysis include a lack of evidence for several variables which are established as influential, particularly on soil N_2O emissions. These include soil aeration, soil C content, and a measure of microbial diversity or abundance. Feeding these into the model would likely explain more of the differences in the data. While some variables are mediated by, or mediate, microbial effects and therefore microbial effects can be inferred from the significance of these mediators, none were detected in N_2O emissions so a more direct approach would have been useful.

6.5.6 Suggested future research

It is important, in a world where emissions reductions are global and national targets for mitigating the effects of climate change caused by GHG emissions, to ensure all options are being explored. This includes less intense methods of production, but also in how to reduce production and consumption when the need for supporting ecosystem service provision meets food demand meets growing human population. Due to the low data availability on semi-natural systems, conclusions drawn from these studies may be less reliable because of a lack of evidence to compare them to. Therefore, research should incorporate a wider variety of production systems when analysing the position soil might play in reducing total emissions. Also, wider research on dietary and cultural preference would be a useful source of information for designing policy and processes spanning the transition to a low-emission or carbon neutral economy.

7: Conclusions

7.1 General discussion

Carbon is a crucial element in the health and fertility of soils, which underpin all life and play a crucial role in human history and productivity (Kutsch et al., 2009). Agriculture has influenced many technological, scientific, social and cultural advances of the last two centuries, from mechanisation, to nutritional health increasing productivity and life expectancy, to the globalisation of the food trade (Gollin et al., 2005).

Agricultural management, crucial for food production, causes SOC reduction and contributes to climate change. Around 8% total carbon stocks stored in the world's soil is estimated to have been lost over the total period of human agriculture (Sanderman et al., 2017), equivalent to 10-20% the total carbon emitted into the atmosphere since the industrial revolution (Dunne, 2017).

Reduced SOC means less C for plants to assimilate and partition into aboveground and belowground biomass; a lack of root stability and aeration exacerbates soil degradation, accelerates soil erosion and reduces net primary productivity (Rattan Lal, 2010a).

The systems by which carbon is stored are crucial to making informed management decisions, but they are not well understood, with two main approaches to quantifying (and particularly to modelling) soil C. Carbon pools describes carbon as lingering in one of several forms of different reactivities, which have varying accessibility in the natural cycle of C. Steady state is reached when SOC remains stable under continuous soil management practices and constant C input levels, which balance the overall C against SOC stocks and SOC mineralisation (Nicoloso et al., 2016). The steady state theory still assumes carbon is in pools, which vary in turnover rate with recalcitrance (Cole *et al.*, 1993). However, carbon pools do not assume steady state. The assumption of constant behaviour does not necessarily indicate that the outcome is equilibrium (Andrén et al., 2004). Steady state theory is an important factor in determining the ultimate capacity of a soil at landscape scale to advise management, to ensure realistic projection of capacity and efficient management (Nicoloso et al., 2016).

The pools theory is an important approach to in this thesis, where C is split into pools of reactivity to analyse changes in recalcitrance as well as total C. While steady state theory is an important perspective to maintain when analysing long-term C changes, it is important to understand short-term differences in C recalcitrancy which respond to management. Timescales of various management techniques and how long they take to have a positive impact on soil C are variable, not only for how much total C might be stored in the soil, but on how accessibly it is stored and therefore how vulnerable it is to being lost to the atmosphere.

Many approaches to improving soil health are already being explored, with substantial evidence that forestry can rehabilitate soil subject to contamination, compaction and carbon degradation (Ward et al., 2021). Additives and inputs including compost, green waste and biosolids are established as C- and N-positive for slowing or reversing soil degradation (Ward et al., 2021). Leading the way are studies in arid soils, which already face issues likely to become more prevalent under climate warming scenarios. These include irrigation to encourage microbial cycling (Entry et al., 2002), reducing tillage to improve water filtration and reduce erosion (Brown et al., 2021; Triplett & Dick, 2008), and biochars or organic mulch to stabilise SOC and reduce run-off (Ding et al., 2016; Ingold et al., 2015). Cover cropping has proved effective in absorbing water and nutrient run-off and preventing soil erosion (Ruiz-Colmenero et al., 2013), and this is a key intervention examined in this thesis.

The approaches address in the WG1 and WG2 experiments incorporate not only species diversity, evidenced as improving soil C storage (Cooledge et al., 2022), but also cover cropping to prevent bare earth (compared to bare control plots). The approaches in the Leicestershire experiment were designed to exclude diversity and the herbal element, but investigate the gains in soil C made under different grass cultivars. The mesocosms were no-till and the Leicestershire experiment had a tillage legacy effect. The application of OM was much more evident in the WG1 mesocosm than WG2, enabling comparisons of OM levels over soil carbon fluctuations and other baseline parameters. Grazing also had a legacy effect on the Leicestershire experiment, and while this could not be replicated in the mesocosms, Chapter 6 builds on the potential changes driven by grazing pressure which could not be investigated *ex situ*.

While some information with an established argument was incorporated into the experiments in this thesis, there are also relevant gaps in the literature, so it was necessary to address these in the experimental design. These are mainly centred around time and depth. This is because soil research is often limited to plough depth, which leaves the potential for slow-forming, recalcitrant or deep carbon untapped. The processes by which C becomes recalcitrant are not particularly well understood, and the timescale by which C recalcitrance changes has little evidence, although there is some evidence that a relationship between depth and recalcitrance exists (Lorenz and Lal, 2005). Storing C in recalcitrant forms is desirable as this removes C from immediate environmental catalysts. Understanding influential plant species, depths or timescales for manipulating C into less reactive forms is an important goal when there is such a variety of grassland and grazing types, a huge potential resource for storing carbon underground.

Time is another aspect to manipulation, given that there is a huge area of temporary grassland in the UK, used in rotation with other crops and for pasture. Over 97% of these have been established for five years or less (DEFRA, 2021b). This means that the majority of research, where long timescales are usually desirable to form a better picture of long-term changes and ensure the reliability of the data, are not particularly applicable to this swathe of grassland. Managements proven to grow SOC stocks over decades are not necessarily appropriate to grasslands which are in and out of crop rotation. Legacy effects are also a vastly under-researched area, because the length of time any benefit to soil health is maintained under cropland is virtually unknown.

There is also very little research on different plant species or functional groups which might be used to manipulate soil C over short periods of time. While legumes are the focus of much research (Cooledge *et al.*, 2022), and highly productive grasses such as perennial ryegrass or kikuyu (Thomas *et al.*, 2012; Grace *et al.*, 2019), there is much less research on herbs despite evidence supporting ley diversity as beneficial to animal and soil health (Brunetti, Morris and Keilty, 2006).

Therefore, this thesis is a three-pronged approach to soil health, focusing on the knowledge gaps of i) time, particularly with regards to very short ley lifespans; ii) depth, because it could hold a potential key for storing carbon away from environmental influences which keep it bioactive; and iii) functional diversity, because of differences in plant biology and chemistry which might be useful for manipulating soil carbon. All of these are framed with the carbon pools theory in mind, because the goal is not only manipulating total C, but on storing it in a pool least vulnerable to environmental change.

7.2 Scope for grassland manipulation as a tool for soil health

Water is an important driver of total C and most fractions of C across almost every experiment in the four experimental chapters. Water is likely to be a powerful driver of soil health, but from historical legacy and the generally good health and high-C content of British soil naturally, water is not a primary concern. In dry periods and drier regions of the UK as the effects of climate change are felt more regularly, irrigation may be necessary as a tool to support soil health and C assimilation into soil (as evidenced from semi-arid soils) (Denef *et al.*, 2008).

As already acknowledged by Natural England and DEFRA in their guidance and recommendations for seed mixes, there is an important role in leys for N-fixing legumes. Grasses rely more on N synthesised by legumes, rather than N available in the soil (Dhamala *et al.*, 2017). Soil N also has an established relationship to SOC. Fertilisers have greatly increased biologically available N in the soil, which may help plants sequester more C in their tissues and partition resources to invest more in belowground rhizodeposition of biomass and exudates, as they overcome the usually limiting factor of N supply (Loubet *et al.*, 2011; Crowther *et al.*, 2019). However, linking this to the findings in Chapter 6, increased N inputs also increase soil efflux of the three main agricultural contributors to GHG emissions (Dalal *et al.*, 2003; Laubach *et al.*, 2016). It is more likely that excess artificial fertiliser would bring about a negative effect than the slow, natural release of N by legumes, and therefore maintaining a proportion of legumes in all leys while reducing inputs is likely to increase the capacity of the soil to hold and build C, in both plant and microbial biomass.

WG1 already had high N and OM content prior to the experiment being planted, and legumes were in their own pots with no crossover between legumes and grasses or herbs. There can be no link in WG1 between legume-derived active soil N and increased C in the pots of any other species than white clover. Lower OM soil in WG2 also coincided with a shorter experiment, meaning it is difficult to separate the effects of time and N on C. N was not a significant driver of C in WG2, but it is likely that the processes governing recalcitrant C accumulation did not reach equilibrium in the one-year experiment. The comparison between WG1 and WG2 indicates that N can start to become a significant predictor of recalcitrant C in as short a period as two years. This evidence suggests that at least two years are necessary to drive a significant C-N relationship, and therefore a ley establishment for two years or more is likelier to influence C storage (and N storage) than leys established for less than two years.

Optimising N supply in low-C croplands can help to reduce C emissions and even reverse the effect into net C storage over six years, (Ammann *et al.*, 2009). As addressed in Chapter 6, N excess can be lost to run-off or leaching, or cause die-off in methanotroph populations, which causes net CH_4 efflux (Prem, Reitschuler and Illmer, 2014). However, targeted mineral N fertilisation can reduce microbial respiration rates, reducing the loss of CO_2 (Lee and Jose, 2003). Mineral N fertilisation has been found to produce a lower microbial respiration rate than organic matter N in the form of cattle manure, possibly because of the added effect of manure to increase soil pH, which has an impact on respiring microbiota (Enwall *et al.*, 2007). High baseline N in WG1 indicates OM inputs, but this imbalance was addressed by using much lower OM soil in WG2 to assess the differences. In WG2, the timothy + red clover combinations produced the effect of storing an increased proportion of soil C at greater depths, although still produced a net loss of soil C overall; this supports the evidence discussed above for longer ley establishment, but also suggests that the inclusion of a legume might reduce losses of C and influence storage at depth. If concentrations of N and C were lower in the soil profile, they would be less vulnerable to atmospheric and environmental pressures, which may allow them to persist longer in the soil.

WG1 did provide evidence of a specific species influencing labile soil C. Yarrow was not expected to be a major driver of soil C, in that it has shallow roots and was generally the smallest herb out of all pots. However, its effect on soil C is not related to its observed aboveground biomass. Belowground root mass in yarrow was very consistent, having more root at depth even than chicory. However, yarrow drove differences only in labile C. Ribwort plantain, a mid-deep rooting herb, also influenced shallow and labile C in WG1, and had a significant influence on mid-lability C in WG2. There is evidence in WG1 supporting a relationship between lability and shallowness, but not a corresponding relationship between depth and recalcitrance in WG1. WG2 provides slightly improved evidence of increasing recalcitrance with depth in ribwort plantain, a mid-rooting species. Therefore there are individual species effects on depth, lability and total C storage, and yarrow and ribwort plantain are suggested as important herbs to include in a diverse ley for improving SOC stocks.

Species diversity did not show as a dominant factor in total LOI in WG2, despite established evidence that plant diversity does improve soil function including C sequestration potential. Herbal leys are a generally accepted method of sequestering more soil carbon than arable crops (Jordon *et al.*, 2022). A diversity of plants produces better aggregate structure, and perennial untilled fields support different groups of microorganisms (Hirsch *et al.*, 2017). Deep rooting plants create deeper rhizodeposits and deeper pore spaces for air and water (Cooledge *et al.*, 2022), which may increase C assimilation at depth by altering pH and water content to a more hospitable environment for a microbial community (Dodd *et al.*, 2011). While patterns were not uniformly significant, species diversity in WG2 did improve root biomass and root depth, produce a slight increase in total weight loss on ignition, and an increase in the proportion of C stored in the recalcitrant fraction. In addition, hybrid diversity (not even functional or species diversity) was indicated as a positive influence on increasing SOC stock in the Halstead experiment, because different cultivars effected change in different C parameters (depth, recalcitrance, overall C gain). This indicates that, given time, a positive relationship between root biomass, depth and increased total or recalcitrant C might develop and that allowing longer establishment times may produce a more prominent diversity effect on C.

Most C losses in the mesocosms are coming from the labile fraction. Given that recalcitrance is the preferred C pool, and ligneous tissues have been linked to more recalcitrant C when broken down, the use of ligneous plants might also be a useful tool in ley manipulation. This could be effective if combined with other treatments, for example ploughing in as a cover crop, or, if tillage is to be avoided, soil flipping or treading in for a ligneous green manure. These ideas are not directly evidenced by WG1 or WG2, given that major drivers derived mostly from the labile fraction. However, additionally managements could utilise some of the evidence in the literature, if further research is designed to address these questions around the effectiveness of multiple approaches to increasing recalcitrant C.

As most changes are driven by the labile fraction, and most of the labile fraction is found in the top 10 cm of soil, the top 10 cm of soil must be an important target for C conservation. Cover cropping and reduced tillage are commonly utilised approaches, but these should be subject to further research to ensure that labile gains are not immediately lost to biomass harvest, for example, and to combine management strategies to analyse which is the most effective combination for maintaining or increasing soil C (e.g. initial changes including cover cropping or mulching, followed by soil flipping or ploughing). The evidence in the mesocosms suggests there could be carbon storage potential in double-stranded management approaches, which first take account of physical soil health by root stability and reduced erosion, and then by OM introduction to deeper soil horizons.

The labile fraction was also linked with other depths in WG2, increasing at lower depths under plant combinations with a timothy partner particularly. This is a positive result, because despite the C remaining in the labile fraction, removing it from common environmental disturbances such as plough range, wind erosion or grazing at the surface is more likely to allow the C to undergo processes influencing recalcitrance over longer periods of time. Depth did not show as a significant predictor in WG1, apart from at 10 cm. This is likely to be linked to time, assuming it takes much longer for OM to be pulled into deeper subsoil layers by soil mesofauna and microfauna, and broken down by a less abundant microbial community. Depth was never a driver of recalcitrant C in either of the mesocosms, providing no evidence in support of the depth = recalcitrance theory. Depth is also not a significant driver of total loss on ignition or any C fraction in WG2, although this may also be influenced by lower baseline nutrient content and shorter establishment time than WG1. However, the Halstead experiment produced depth as a significant predictor of every fraction of C, and total C. This suggests that the longer a ley is in place for, the more likely a depth effect (even if just at 10 cm) is to become evident in terms of C concentration differences. Allowing a longer establishment time for leys, where possible, may influence greater C storage.

Time was a significant predictor of soil C changes, including in recalcitrant C changes in summer 2020 after the first growing season of WG1. Recalcitrant C does tend to accumulate over time in WG1, even if an initial drop is experienced when plants in a growth phase are resource-demanding. No sample month showed as a significant predictor of recalcitrant C; however, in WG2, patterns were observed indicating PCT and TP as likely combinations to increase the most recalcitrant forms of C over a period of less than a year. This must be treated with caution because it is undetectable in the GLM and has a large SE, indicating a variable effect. However, time is indicated as an important driver in WG1 and WG2, and therefore a key tool in encouraging grasslands to sequester more carbon would be to leave them in place for longer. The recommendation for the length of time would be field-dependent as baseline soil quality will influence the time required to reach a certain C concentration, and other variables including species diversity must also be considered.

WG1 and WG2 show a weak positive correlation between root biomass and SOC, although the Halstead experiment does not reflect this. Despite the relationships being undetectable in a GLM, a correlation suggests that over time or with a greater number of samples, the pattern might become more evident. This is also an easy manipulation to make when seeding a new ley, because deep-rooting mixes can be bought prepared.

Grazing could not be tested in WG1 or WG2 mesocosms, and while Halstead had a grazing legacy effect, this was not discernible because of the lack of time progression. However, the meta-analysis in Chapter 6 investigated the effect of grazing, and found there is little in common between studies achieving GHG sequestration, as they span a range of grazing pressures and climates, and mostly fall in improved/semi-improved grassland categories due to sampling bias. However, the model indicated that grazing produces a higher CO_2 loss from the soil than no grazing, and sheep grazing produces a greater loss of CO_2 than cattle grazing. Higher also LSU increased CH_4 emissions; however, there is a weak negative correlation between N_2O and LSU which is due to one paper and could potentially be the result of sampling bias or error. Overall, the indication from the meta-analysis is that lower grazing pressure reduces GHG loss from soil, and reduced or no grazing helps to retain soil carbon and nitrogen.

7.3 Management recommendations

Funding C leys of individual species is not an effective use of finance directed solely towards improving soil C concentrations, and there is no indication from WG1 that a high concentration of timothy, yarrow, chicory, white clover, dandelion or ribwort plantain, based only on the results of this experiment. However, there is some benefit to establishing a ley in terms of SOC, even if the difference is not significant over two years in an already C-rich soil. Using leys in combination with other managements including limited or no tillage for the duration of the ley and then shallow ploughing the cover crop back in as green manure could be helpful for increasing SOM on a short timescale, such as that of a temporary agricultural ley, which is generally around two to five years. These managements can be used on C-rich soils to prevent further C loss.

WG2 was even more time-limited than WG1, and therefore time may be an even larger contributor to the lack of predicting power in the model. However, it is an important piece of information for forming management and funding plans, that leys in place for less than a year (and according to WG1, less than two years) are not likely to bring significant C benefits by increasing the proportion of C stored in less labile fractions. If an effect on C cannot be observed in one or two years in the mesocosms, they are not likely to have a positive effect on soil C in the field over much larger areas, which are also subject to more change in terms of wind erosion, weathering, tillage, artificial inputs, grazing or harvest, and legacy effects of degradation of soil structure and fertility. Therefore, I would recommend that leys be in place for two years or more from the results of these two mesocosms, and potentially even longer if possible.

That no species or combination achieved a net gain in SOC over the nine-month period of WG2 is important. This should inform policy which subsidises one-year leys, given the approach to Environmental Land Management Schemes (ELMS) being brought in in the UK from 2023 which include an incentive for improving soil heath. The WG2 mesocosm shows that one year is not enough time for herbal ley growth alone to improve soil health. It may have benefits alongside other management strategies, but time is likely to be a key player in the effectiveness of encouraging plant biodiversity for its C-storage effects. I would recommend that leys should not be subsidised under ELMS unless they are in place for two years or more, if one of their goals is improving soil health.

It is also the case that solely soil-derived C benefit is not the total benefit from herbal ley cover cropping, but also that cover cropping alone is unlikely to achieve significant soil C gains over two years or less. Increasing soil C by plant root biomass and rhizodeposition, which thereby support a healthy microbial community (on a longer timescale than WG2) which process soil C and store it in microbial biomass, are important initial strategies. However, adding to this by potentially incorporating the cover crop as green manure by sacrificing a hay harvest would also mean reduced fertiliser inputs, which come with their own carbon cost in terms of production and transport. Given evidence in support of cover cropping and associated managements such as green manuring in the literature, I would suggest that the results presented in this thesis might support development with additional management strategies.

There is not enough evidence in the Halstead experiment to provide a recommendation of any one cultivar to have a consistent positive effect on total soil C, or any fraction of soil C, similar to WG1 and WG2. No significant relationships were established with root biomass as a driver of soil C and consequently, any species-driven differences in LOI values are being driven by an indirect effect of a variable not included in the model. Root biomass effects on C fraction are cultivar-specific, and not a general rule across Festulolium cultivars. Without a baseline, it is impossible to tell whether the cultivars have had an overall positive effect since the ley was established. They can only be compared to each other. The three strands of targeted soil C improvement are all met by different cultivars: i) storing C at depth, away from environmental interference, has the strongest response to Fojtan; ii) storing C in recalcitrant forms, so it is less reactive to stimuli and remains in the soil, has the strongest response to Aberniche; and iii) storing more C by % weight in the soil has the strongest response to Broadsword or Lofa. That different cultivars respond to each of these targets is simply evidence to support the theory of diversity in a ley, than evidence supporting any one cultivar as a panacea for carbon sequestration. Therefore, the only definitive management recommendation for improving every aspect of soil C improvement is increasing diversity in grassland leys.

The grazing meta-analysis indicates that reducing inorganic inputs inhibit methanogenesis by altering the abundance or functional groups in the microbial community. Therefore reducing N applications should limit methane and carbon dioxide efflux from the soil. Maintaining soil moisture at a level which supports bacterial growth but does not flood the soil to the point of creating anaerobic conditions is also likely to have a positive effect on emissions reductions, which, in the UK, is likely to be irrigating pastures only in drought. Reducing LSU, because grazing pressure influences soil OM content and the partitioning of plant resources into belowground biomass, may also reduce soil C and N loss from soil. Sheep drive higher soil CO_2 emissions than cattle and therefore should be avoided. This evidence also suggests that a substantial driver of emissions is the state of grassland being ‘semi-improved’, which could relate to a high input but lower LSU. Therefore semi-natural grasslands in particular should receive targeted inputs, if any, and reduced grazing pressure to try to mitigate some of the C and N flux from the soil.

7.4 Conclusions

Grasslands are under a range of pressures, from food production, to climate stressors, to degradation of soil health. However, as a large proportion of both global and British land area, they are an important tool in mitigating climate stress and food insecurity. Temporary grasslands are a long-established part of many agricultural rotations, but specifically using them to improve soil health is a relatively new approach. There is little available research on the establishment periods necessary to achieve net C gain in temporary grassland soils, or on the depths to which this effect can be observed, or the influencing plant species.

This thesis was designed to explore pools of carbon recalcitrance, over a short period of time relevant to a temporary ley, and the depths to which the effects of surface plant species can be observed. Potential impacts of individual species and combinations of functional groups on soil carbon, and other soil parameters which are closely linked with carbon or other mediators such as the microbial community, were assessed in mesocosms allowing changes to be measured over precise depth and time intervals. Root biomass was analysed for any relationship with depth, carbon content or recalcitrance in both the Halstead experiment and the mesocosms. Investigating Festulolium hybrids on an experimental ley in Halstead was designed to assess rooting morphology in relation to C and other variables, as well as filling in a knowledge gap around in situ leys under grazing pressure. Finally, incorporating a grazing element in the thesis was important, as supporting livestock is the main purpose of maintained grasslands globally. Throughout these chapters, the complex interactions of soil physical and chemical parameters, communities and processes are explored, adding to the information available on the impacts of leys on soil C, and how management strategies can mitigate C loss.

With exploration of these influencing variables, further questions became evident, particularly around underrepresented grassland-grazing systems, microbial influence, and the potential legacy effect once a ley is returned to arable rotation. There is still much to be learned around carbon-influencing processes, especially on short-term leys, but this research has made some contribution to our knowledge on the time, depth and species effects influencing changes on soil carbon.

Word count: 82,901

Appendix 1

Table 1. Each reference and the database(s) which returned it, and the justification for rejection or acceptance into this review alongside the stage at which the paper could be eliminated

Database(s)	Reference	Stage at which paper was eliminated	Justification	Accepted
BioOne	2013 Canadian Society of Animal Science-Canadian Meat Science Association Joint Meeting, 2014	Abstract	Full article inaccessible; also discounted for its authorship by the Meat Science Association	No
BioOne	Abstracts from the Annual Meeting of the Kentucky Academy of Science 2–3 November 2019 Berea College, 2020	Abstract	Inaccessible via link provided; no full reference returned so cannot search other databases	No
Scholar	Allard et al., 2007	Eligible	The study is based in upland semi-natural grassland in France, with comparable agricultural system and climate, including rainfall (1200mm)	Yes
Scholar & WoS	Allen et al., 2009	Title	Because of the focus on afforestation, returning pasturelands to forest, this is not applicable to UK agroecosystems	No
Scholar	Assouma et al., 2019	Title	Taking place in a semi-arid climate, this is not an applicable dataset to use with regards to the UK	No
BioOne	Baldock et al., 2012	Abstract	Focus on how heat and drought increase in frequency and magnitude would affect soil carbon stocks in Australia. Focus and climate irrelevant	No
WoS	Beauchemin et al., 2010	Eligible	Measuring total greenhouse gas emissions over eight years in a climate comparable to the UK. This does however involve feedlots and barn, so pasture data will be selected for the review	Yes
WoS & Scholar	Beauchemin et al., 2011	Eligible	This primary study includes the whole cattle life cycle, including feeding	Yes

			them on native pasture in a climate and rainfall not too different from the UK	
BioOne	Bedard-Haughn, 2011	Abstract	Not relevant to grazed pasture gas flux or livestock emissions	No
WoS	Bell et al., 2012	Abstract	Here the focus on climate change effects (particularly drought) in an Australian livestock system is not comparable to British grazed grassland	No
Scholar	Bellarby et al., 2013	Methods	This is a pan-European study on a scale too large for this review, and the greenhouse gas emissions discussed are unrelated to grassland and policy focused	No
Scopus	Berchielli et al., 2012	Title	Tropical pastures would mean the input to the enteric emissions is quite different as dietary effects can be significant (Humphreys *et al.*, 2017; Dangal *et al.*, 2020)	No
Scholar	Bergier et al., 2019	Abstract	This research focuses on Brazil, in a region of the world's largest tropical wetland area, and the world's largest flooded grasslands.	No
BioOne	Bhochhibhoya et al., 2017	Title	Set in a mountainous area, not upland/lowland/ pasture; focusing on building material analysis not grassland; and assessing global warming potential rather than gas flux measurement	No
WoS	Biswas et al., 2010	Abstract	This is on a combined farm, with intensive sheep rearing which is generally at odds with the sheep rearing system of the UK, in a very different climate, soil and input regime	No
WoS & Scopus	Boon et al., 2014	Eligible	Because this is a study on British lowland peat soils, which are currently underrepresented in this	Yes

			literature, the gas fluxes under excreta treatments may be extrapolated for field-wide data and could be useful in this review.	
WoS & Scopus	Bošnjak et al., 2018	Abstract	This is not an experimental study providing primary data, and its focus is on mitigation for policy	No
BioOne	Brock et al., 2012	Methods	Two-fold inapplicability: set in New South Wales, this study follows the total life cycle emissions of a tonne of wheat. No climate or livestock relevance	No
WoS	Cai, Chang, et al., 2017	Methods	Because it is a review, and focused on excreta patches rather than holistic emissions, data from this cannot be incorporated into the review as its measurement is incongruous with other papers and should also be taken account of within entire grassland emissions in other literature	No
WoS	Cai, Du, et al., 2017	Title	The subject, the climate, and the farming system are all too far removed from the UK system to be relevant	No
Scholar	Cezimbra et al., 2021	Abstract	The study area is in the Pampa, which is subtropical in the north and semi-arid in the west and south	No
PubMed, Scopus & WoS	Chang et al., 2021a	Methods	The data is not primary, no experimentation has taken place. This follows global trends which are too far extrapolated to be relevant to UK pasture	No
BioOne	Chapman et al., 2012	Title	Another Australian paper focusing on abiotic crop stressors and other climate change impacts on crop productivity, and how crops may be adapted to these by breeding. Not	No

			relevant to British soil carbon.	
WoS	Charteris et al., 2021	Eligible	The methods of this experiment are robust, covering three main greenhouse gases, and the study takes place in Britain	Yes
Scholar	Chen et al., 2017	Title	The semiarid steppes are too different for comparison to UK grasslands	No
PubMed & WoS	Chiavegato et al., 2015	Eligible	Based on land which was under deciduous forest prior to 1860s, in Michigan where the climate is not wildly different to the UK because there is not a significant wet/dry season. The soils are well-drained sandy loam, with forage cover at 80% grass and 20% other herb like UK pasture	Yes
Scholar	Clark et al., 2001	Abstract	This report focuses on the management practices and technologies to reduce GHGs, not measuring them	No
BioOne	Cook et al., 2010	Abstract	The focus in this paper is on managing sources and sinks of greenhouse gases in tropical savannah and semi-arid rangelands of Australia; not relevant to the temperate climate or livestock production system of the UK	No
BioOne	Cowie et al., 2012	Methods	This is an extremely complex global review encompassing different climates, frameworks, and national and international organisations and targets, and is therefore not applicable to the UK	No
Scholar	Cunha et al., 2016	Abstract	A study in the context of a tropical climate in Brazil, specifically experimented because the climate is so different from previous	No

			studies Brazilian farm design has relied on	
WoS	Dalal et al., 2003	Methods	This is both a review, and Australian, with incomparable climate and land management	No
Scopus	Dangal et al., 2020	Methods	While useful to reference, the data is not specific enough to include in this systematic review; it is a wide-ranging global review paper	No
Scholar	De Klein & Ledgard, 2005	Methods	This study focuses only on N_2O and how to mitigate its contribution to New Zealand emissions	No
Scholar	De Klein et al., 2008	Methods	This paper is much too broad to be applicable to the conditions of this review	No
Scholar	De Klein et al., 2010	Abstract	This is a review and directed towards future research	No
Scopus & Scholar	De la Motte et al., 2019	Eligible	A study based in Belgium, with a similar climate to the UK, similar soil fertility, and standard European nitrogen application and representative stocking rate	Yes
Scholar	Del Prado et al., 2013	Methods	This paper analyses the limitations and strengths of the different existing approaches for modelling greenhouse gas mitigation	No
WoS & Scopus	Dengel et al., 2011	Eligible	This Scottish study examines sheep grazing, which is currently underrepresented in this review, and the effect of their grazing using eddy covariance	Yes
Scholar	Dick et al., 2015	Title	A humid tropical/sub-tropical climate, which rarely drops below 14° C, and a soil history and forestry vastly different to UK mean this study cannot be used in this review	No
BioOne	D'Odorico et al., 2010	Title	Ecohydrology, not carbon biology in pasture	No

WoS	Drewer et al., 2017	Eligible	A British grazed grassland, but the focus of the study is on the effect of ploughing. Therefore, in this review only the control (no ploughing) data will be used	Yes
WoS & Scholar	Dumortier et al., 2017	Eligible	Set in Belgium, this study takes place in a climate similar to that of the UK. Its focus on both cattle emissions and pasture emissions makes it very relevant	Yes
PubMed	Duval et al., 2013	Abstract	Changing over to an energy crop is not maintaining consistent pasture, so cannot be used in this review	No
BioOne	Eckard et al., 2012	Abstract	Review of future challenges, not primary data/gas flux focused	No
Scholar	Eckard et al., 2018	Title	This is not primary data or an experiment, and has a target of Australian policy unrelated to the UK	No
BioOne & WoS	Finn et al., 2014	Abstract	No experiment for primary data; climate not comparable to UK	No
WoS	Flessa et al., 2002	Eligible	Set in a comparable climate and farming system in Germany, which is geographically close to the UK with comparable climate, and additionally under CAP policies and similar stocking densities	Yes
BioOne	Follett & Reed, 2010	Abstract	Without primary data, the focus of this study is discussing soil carbon sequestration benefits and specifically US policy impacts, no relevance to gas efflux from British grazed grasslands	No
Scholar	Gaitán et al., 2016	Abstract	With the conditions of this study comprising a tropical climate in the Caribbean, where temperatures average 20-30° C all year round, this is not an	No

			appropriate study for this review	
Scholar	Garnett et al., 2017	Abstract	This is a very large international report which is a kind of review examining many different livestock systems	No
BioOne	Gemeda et al., 2014	Title	Two-fold inapplicability to the UK, by 1) focus on digestibility not emissions, and 2) of tropical grasses	No
Scholar	Gill et al., 2010	Title	This study is a wide-ranging review which concentrates on breeding and feeding of livestock	No
BioOne	Gilmanov et al., 2010	Title	This paper is too broadly directed, with world data reviewed (not primary) and a focus on productivity not gas efflux	No
WoS	Giltrap et al., 2010	Methods	This paper is analysing the process and outcomes of a model and the only validation performed on grazed pasture only accounts for N_2O	No
PubMed	Gomez-Casanovas et al., 2018	Abstract	Study of tropical and subtropical areas in a wet-dry seasonal cycle only, very different to the UK and these pressures have a significant influence on soil carbon	No
BioOne	Gosnell et al., 2011	Title	Rancher engagement in climate change mitigation strategies. No primary data/UK applicability	No
BioOne	Goss, 2013	Abstract	A critique of climate change drivers, ethics and consequences behind soil productivity, as a preface or accompaniment to a book. This is not relevant to livestock grazed carbon balance in the UK.	No
Scholar	Graux et al., 2011	Methods	This paper focuses on livestock by weight, not holistic emissions data	No
BioOne	Groffman et al., 2012	Title	A US forest-based study, irrelevant to British livestock pasturing	No

WoS	Guo et al., 2015	Title	The Tibetan plateau alpine meadow ecosystem is too far removed from British climate and soil conditions	No
Scholar	Hammar et al., 2022	Eligible	Although not the entirety, forage-fed animals are considered. This is modelling, taken from existing data, so while it isn't primary research, the data is Swedish (comparable conditions to the UK) and has added value via the model output	Yes
WoS	Hartmann & Niklaus, 2012	Methods	The main focus here is the drought effect and fertiliser application, not the presence of livestock on a pasture	No
WoS	Henderson et al., 2017	Methods	This focuses on pricing per metric ton of emissions reduced, not primary research on the emissions themselves	No
BioOne	Henry et al., 2012	Abstract	An Australian paper taking in drought, heat, feed crop scarcity, disease and pest distribution but which is not relevant to British grassland efflux or concordant cattle or sheep emissions	No
Scholar	Herrero et al., 2016	Abstract	A review of the potential of management options for mitigating greenhouse emissions in livestock production, this is not appropriate for this review	No
WoS	Hiltbrunner et al., 2012	Abstract	This is not an agricultural flux focus, but on afforestation in Norway	No
WoS	Hörtnagl et al., 2018	Eligible	Set in central Europe and thereby comparable to the UK if drier, this is an experiment using the eddy covariance technique on N_2O, CH_4 and CO_2 in grazed grasslands	Yes
BioOne	Hristov et al., 2011	Abstract	A review, too broad-ranging, which focuses on the ammonia emissions of	No

			intense feedlots and not soil carbon flux or enteric emissions	
WoS	Hu et al., 2018	Methods	This study of Tibetan alpine meadow reflects conditions too far removed from British livestock farming to be useful in a British-based review	No
Scopus	Humphreys et al., 2017	Methods	This paper is specifically analysing the impact of white clover in pasture fields and how it lowers the carbon footprint per litre of milk. The whole book chapter is also unavailable via University of York library	No
Scholar	Huth et al., 2012	Eligible	This fenland in Germany relates to low-intensity wet grasslands such as those in Norfolk and north Devon, which are underrepresented in the literature, in a comparable climate and geographically close	Yes
Scholar	Iwaasa & Lemke, 2014	Methods	This is a focus on management, with no primary data	No
BioOne	Iwasa et al., 2015	Title	Japanese study focusing on coprophagous insects, so N and C measures are taken, but directed only on emissions from individual deposits; the study was in vitro not in the field; and results focused on insect population which cannot be made uniform (very scarce information) in this review	No
BioOne	Jayasundara & Wagner-Riddle, 2014	Methods	A review without a primary experiment, using milk yield data and International Dairy Federation GHG signature data to allocated gas emission per kilo of milk.	No

Scholar	Jérôme et al., 2014	Eligible	This is an experiment with primary data, and climate, soil and cattle raising system comparable to those of the UK. However, only carbon dioxide is taken into account	Yes
Scholar	Jonker et al., 2018	No access	No access through institution/other methods	No
BioOne	Joyce et al., 2013	Abstract	North American rangelands are not particularly well comparable to UK grasslands, and the focus of this paper is on assessing mitigation and adaptation, not primary data on gas efflux under grazing	No
WoS	Kirschbaum et al., 2013	Abstract	This study focuses on the shift from agricultural land to forest, or vice versa	No
BioOne	Lal, 2010	Abstract	This paper is too broad for the focus of this systematic review, covering global environmental and economic benefits of soil conservation	No
WoS	Laubach & Hunt, 2018	Eligible	With a comparable climate (New Zealand) on well-drained soils with perennial ryegrass and clover pasture, as found in most UK pastures	Yes
WoS	Laubach et al., 2016	Eligible	The data on irrigated pasture can be used, as NZ has a temperate climate although somewhat drier than the UK.	Yes
Scholar	Lawton, 2005	Eligible	This is a very useful paper applicable to this review, set in the Irish climate and grassland system, collecting primary data with rigorous methods	Yes
BioOne	Legesse et al., 2018	Title	Cattle diet manipulation study, not relevant for UK pasture fed cattle	No
Scopus	Liang et al., 2020	Eligible	The diet of these cattle is largely forage-based, and	Yes

			enteric and manure CH4, NH3, and soil N2O are all accounted for	
WoS & PubMed	Liebig et al., 2010	Eligible	This primary data study has a relatively comparable climate and is a comprehensive study	Yes
BioOne	Liebig et al., 2014	Title	Refers to semi-arid rangeland and therefore isn’t applicable to UK climate, soil or livestock production	No
Scholar	Lin et al., 2009	Title	Based on the Qinghai-Tibetan plateau, the species, systems and climate are inapplicable to the UK	No
BioOne	Lipper et al., 2010	Title	On ecological and economic returns for improving soil carbon in West African rangelands; no primary data or UK applicability	No
WoS	Liu et al., 2009	Title	Inner Mongolian steppe is too different in climate, soil, and livestock production system to the UK	No
WoS & Scholar	Ma et al., 2006	Title	Because of the extremely cold climate (-15 to -30° C in winter) and the harsh environment of the steppe, this is not comparable to UK climate and farming systems	No
Scholar	Manono, 2016	Eligible	Although there is a focus on effluent dispersal, some of the primary data is useful in this review due to the comparable climate and soils. Despite <1 month sampling time, it took into account the entire 17-day irrigation cycle	Yes
WoS	Mazzetto et al., 2015	Title	Brazilian high intensity ranch farms are not comparable to British grazing because of climate, environment, and land use change impacts	No

Scholar	McGilloway, 2005	Abstract	No access, and it is a book, not a paper, with no primary research	No
Scholar	McGinn et al., 2014	Eligible	A thorough primary study of cattle-grazed grassland comprising N_2O, CH_4 and CO_2 measurements from both animal and soil origin	Yes
WoS	Mei et al., 2018	Title	Due to the semi-arid climate where mean annual rainfall ranges from 350 to 450 mm and mean annual air temperature ranges from 5.8 to 7.3 °C this is not applicable to UK system and climate	No
WoS	Meier et al., 2020	Methods	The Perth climate (Australia) is too hot to be applicable to British soils, as climate and weather conditions do affect the soil carbon (the coldest average temperature in the vicinity of the study is 13° C, the soils are acidic, rainfall is a fraction of the UK average, and there is some controlled burning on pasture)	No
BioOne	Mendelssohn et al., 2012	Title	Southern US coastal wetlands and oil damage, not related to UK pasture	No
Scholar	Motha & Baier, 2005	Abstract	This paper focuses on the impacts, not measures or data, of climate change, not soil flux, in North America.	No
Scholar	Nguyen et al., 2012	Methods	This study is not based at all on grassland grazing, but on intensive maize feeding	No
WoS & PubMed	Oduor et al., 2018	Title	Subtropical rangeland in Kenya is not applicable to the consistent pressures on UK soil or the climate from livestock grazing	No
BioOne	Oenema et al., 2014	Abstract	Focus on drivers and constraints of grassland and forage intensification, not measuring gas fluxes	No

Scholar	Oliveira et al., 2020	Title	As this study takes place in a tropical climate, it is not applicable to the conditions in the UK	No
Scholar	Pelster et al., 2016	Title	The cattle (Bora), system (calliandra tree fodder) and climate (east African) are not comparable to the UK	No
WoS	Peterson et al., 2021	Methods	The focus is on native prairies with different grass species which are not grown in the UK, and which influence CH4 enteric emissions; also, the humid subtropical environment of temperatures is much higher than the UK	No
Scholar	Pinares-Patiño et al., 2011	Methods	This is not a well-referenced paper, and not a primary study	No
Scholar	Piva et al., 2019	Title	Based in a subtropical climate, the conditions of the gas flux are very different to those in the UK	No
WoS	Radrizzani et al., 2011	Abstract	This study is researching a small fast growing tree crop in an Australian state with average temperatures of 30° C so this is not applicable to UK livestock grazing	No
WoS & Scholar	Ramírez-Restrepo et al., 2019	Abstract	Neotropical savannah and tropical climate is irrelevant to UK conditions; additionally, this is focused on animal performance and not carbon balance	No
Scholar	Ramírez-Restrepo et al., 2020	Abstract	The study is based within a tropical climate on neotropical savannah in Colombia, not translatable to British soil and system	No
WoS & Scopus	Raposo et al., 2020	Abstract	Regarding the emissions from tropical Marandu grasslands in Sao Paolo, Brazil, this is also inapplicable to British	No

			grasslands due to grass species and climate factors	
PubMed, WoS & Scopus	Ribeiro et al., 2016	Abstract	The study area is not arable, it applies to Caatinga, a Brazilian native forest type prone to extended drought	No
Scholar	Rice & Rivera-Zayas, 2017	Abstract	No access	No
Scopus	Rivera et al., 2019	Abstract	Based on a tropical dairy, this data is not relevant to temperate UK system	No
WoS	Rong et al., 2015	Abstract	This study is based in temperate semi-arid steppes in China	No
Scholar	Ruviaro et al., 2015	Abstract	The location is in southern Brazil and thereby the climate, soil conditions, ecology are all incomparable to UK conditions	No
WoS & Scholar	Saggar et al., 2010	Eligible	Although this is a review, it is taking data almost exclusively from New Zealand, which has a comparable climate to the UK, and focuses on sheep which are underrepresented in the literature and therefore the contribution of sheep is compensating for non-primary data	Yes
Scholar	Salvador et al., 2017	Abstract	Based on small-scale mountain farms in the Italian Alps, this study is not applicable to the vast majority of UK farming	No
Scholar	Samsonstuen et al., 2020	Eligible	Variability in greenhouse gas emission intensity of semi-intensive suckler cow beef production systems. However, only data from the south-west farm will be used because the temperatures are so low and climate much more harsh than British in the other study areas	Yes
Scholar	Schaufler et al., 2010	Eligible	Based in European grasslands, comparable to	Yes

			UK climate, this is a primary data study	
Scholar	Schils et al., 2005	Eligible	Based in Europe, this is comparable to UK climate and ruminant agricultural systems under CAP and common European pasture grass species	Yes
WoS, PubMed & Scopus	Schils et al., 2013	Abstract	Not a primary data study and only includes N2O. The focus is on mitigation	No
Scholar	Schönbach et al., 2012	Abstract	Steppe is not comparable to the UK grazing system and climate	No
Scopus	Shepherd, 2011	Abstract	This review is too wide ranging for the data to be included in the systematic review; it is not specifically focused on enteric or grassland emissions or experimentation, instead focusing on controversies of these	No
Scholar	Sherlock et al., 2002	Abstract	This examines emissions directly from fertiliser application, not from grazing animals, and not emissions from the total grassland	No
Scholar	Skiba et al., 2013	Eligible	This primary Scottish study is based in sheep-grazed uplands	Yes
WoS & Scholar	Soussana et al., 2004	Eligible	It is a review, but of farms which are all in France. These are applicable to UK systems, because of the European policy framework, and under a relatively comparable climate, if slightly warmer and drier, in France	Yes
Scholar	Soussana et al., 2007	Methods	This is not primary data. Fitting a model to 20,000 published and unpublished French data from the INRA 2002 database, over a very wide variety of agricultural systems, means that data specifically relating to grazed grasslands cannot be picked out.	No

Scholar	Soussana, 2008	Abstract	This is a discussion of the carbon cycle and how agriculture feeds into it, without primary data, without experimentation, and a focus on a very large scale	No
Scholar	Soussana et al., 2010	Methods	No full access, but the data comes from (Soussana et al. (2007) which has been added above, and Soussana et al. (2004) which has also been returned using the Scholar search	No
Scholar	Soussana et al., 2014	Abstract	Because this is a very comprehensive review, the data is not applicable in this British-based review	No
WoS & BioOne	Stewart et al., 2014	Methods	Contains data from analysis of total greenhouse gases from raising beef cattle on pasture, including directly from the soil of the grassland	Yes
BioOne	Sullivan et al., 2015	Title	Archaeobotany to research woodland fire ecology. Not relevant to UK pasture carbon balance	No
Scholar	Taylor et al., 2016	Abstract	*Leucaena leucocephala* is usually grown in the tropics and also used in agroforestry. This studies an irrigated system in a sub-tropical humid climate (Queensland, Australia)	No
PubMed & Scopus	Thomas et al., 2012	Eligible	Despite the Mediterranean climate, this was included because the modelling data for Kikuyu was actually perennial ryegrass data due to similarities in the plant and a shortage of Kikuyu data; and for the paper's focus on sheep, which are underrepresented in a body of literature very focused on cattle	Yes

BioOne	Thomas et al., 2017	Eligible	Used with some caution due to the climate of the study area being significantly drier than the UK, if of comparable temperature, this paper does comprehensively measure pasture gas flux with vented static chambers under low cattle stocking rates	Yes
Scholar	Tully et al., 2017	Title	The climate and systems from Kenyan farms are too different to be applicable to the UK	No
WoS	Uddin & Kebreab, 2020	Abstract	This is a review on the impact of climate change and the food supply system on pastoral industry, not primary greenhouse gas data	No
WoS	Veber et al., 2018	Abstract	The presence of livestock is not a factor in this study	No
Scholar	Vigan et al., 2017	Abstract	This paper is irrelevant to this review because it focuses on livestock mobility and the methods of C4 measurement	No
WoS	Wang, Wang et al., 2013	Abstract	Steppe grazing, in terms of climate, grass species and frequency of overgrazing, is too different to British pasture grazing to be relevant in this review	No
WoS	Wang, Huang et al., 2013	Abstract	No access through institution	No
WoS	Wilson et al., 2011	Abstract	A focus on meat productivity rather than emissions or carbon balance leaves this study inapplicable to this review	No
WoS	Wolf et al., 2010	Abstract	Steppe grazing, in terms of climate and grazing pressure, is too different to British pasture grazing to be relevant in this review	No
WoS	Wu et al., 2022	Eligible	While not a primary study, this paper on a model of the emissions British beef cattle and lamb	Yes

			production which takes in the N cycling, animal intake of forage, grassland emissions, drainage, and excreta to measure N_2O, NH_3, CH_4 and CO_2	
WoS	Yoshitake et al., 2014	Methods	The study species, *Zoysia japonica*, isn't grown in the UK, and outside of Japan is generally only used for golf courses and recreation; the study was conducted in a mountainous area of Japan covered in snow from December-April.	No

Table 2. Converting given units to a common unit

Paper	Given unit	Given weight unit	To common weight unit (g)	Given area unit	To common area unit (m^{-2})	Given time unit	To common time unit (y^{-1})
(Boon *et al.*, 2014)	µg m^{-2} d^{-1}	µg	/ 1,000,000	m^{-2}	-	d^{-1}	x 365
(Chiavegato *et al.*, 2015; Manono, 2016; Drewer *et al.*, 2017; Charteris *et al.*, 2021)	mg m^{-2} h^{-1}	mg	/ 1,000	m^{-2}	-	h^{-1}	x 8760
(Dengel *et al.*, 2011; Laubach *et al.*, 2016; Laubach and Hunt, 2018)	nmol m^{-2} s^{-1}	nmol		m^{-2}	-	s^{-1}	x 31,536,000
(Chiavegato *et al.*, 2015)	g m^{-2} h^{-1}	g	-	m^{-2}	-	h^{-1}	x 8760
(Dengel *et al.*, 2011)	g m^{-2} over 217 d	g	-	m^{-2}	-	217 d	/ 217 x 365
(Dengel et al., 2011)	nmol m^{-2} d^{-1}	nmol		m^{-2}	-	d^{-1}	x 365
(Liebig *et al.*, 2010; Schaufler *et al.*, 2010;	µg m^{-2} h^{-1}	µg	/ 1,000,000	m^{-2}	-	h^{-1}	x 8760

Huth, Jurasinski and Glatzel, 2012; Drewer *et al.*, 2017; Charteris *et al.*, 2021)							
(Boon *et al.*, 2014)	mg m^{-2} d^{-1}	mg	/ 1,000	m^{-2}	-	d^{-1}	x 365
(Hammar, Hansson and Röös, 2022)	mg ha^{-1} y-1	mg	/ 1,000	ha^{-1}	x 10,000	y^{-1}	-
(Flessa *et al.*, 2002; Chiavegato *et al.*, 2015)	kg ha^{-1} y^{-1}	kg	x 1,000	ha^{-1}	x 10,000	y^{-1}	-
(Jérôme *et al.*, 2014)	g m^{-2} d^{-1}	g	-	m^{-2}	-	d^{-1}	x 365
(Thomas *et al.*, 2012)	tonnes ha^{-1} y^{-1}	t	x 1,000,000	ha^{-1}	x 10,000	h^{-1}	x 8760
(Beauchemin et al., 2011; Beauchemin et al., 2010)	mg ha^{-1} h^{-1}	mg	/ 1,000	ha^{-1}	x 10,000	h^{-1}	x 8760
(Stewart *et al.*, 2014)	kg ha-1 y-1	kg	x 1,000	ha^{-1}	x 10,000	y^{-1}	-
(Chiavegato *et al.*, 2015)	mg ha^{-1} y^{-1}	mg	/ 1,000	ha^{-1}	x 10,000	y^{-1}	-
(Schils *et al.*, 2013)	gigagram ha^{-1} over UK grassland	Giga-gram	X 1,000,000,000	4500000 ha		y-1	

Other soil and enteric emissions calculations:

Dengel et al. (2011) used nmol CH_4 m^{-2} s^{-1}

- 0.08 nmol CH_4 m^{-2} s^{-1}
- 1,000,000,000 nmol = 1 mol
- 31,536,000 seconds = 1 year
- = 0.00252288 mol m^{-2} y^{-1}
- = 0.04047513 g CH_4 m^{-1} y^{-1}

Laubach and Hunt (2018) also used nmol for N_2O m^{-2} s^{-1}

- 0.77 nmol N2O m-2 s-1
- 1,000,000,000 nmol = 1 mol
- 31,536,000 seconds = 1 year
- = 0.0242872 mol m^{-2} y^{-1}
- = 1.06876282 g N_2O m^{-2} y^{-1}

Dumortier et al. (2017)

- Enteric emissions given per head
- Used Belgian Blue cattle, average weight of 875 kg.
- Divide head weight for kg^{-1} liveweight emissions

Laubach and Hunt (2018) stated enteric emissions from livestock in ha

- LSU values ha^{-1} were used to give a value of 2.36 LSU emitting 28.76 g CH4 m-2 y-1
- However LSU values are given in ha, so CH4 over ha = 287600 g ha-1 y-1
- = 287 kg over 2.36 LSU = 121.6102 kg CH_4 LSU^{-1} y^{-1}
- Average dairy cow of Holstein/Friesian-Jersey cross (the most common cow breed in New Zealand – the paper does not specify which breed) weighs around 400 kg
- Therefore, LSU value / 400 = CH_4 kg $liveweight^{-1}$
- 304.025g CH4 kg $liveweight^{-1}$ y^{-1} or 0.832945 g CH4 kg $liveweight^{-1}$ d^{-1}

Schils et al (2013) gave grassland emissions as a modelled estimate over all grassland from the UK, and enteric emissions from the whole population of sheep and cattle in the UK, in the year 2000

- Soil emissions in gigagrams from the grassland of the UK
- 4,500,000 ha grassland in the UK (DEFRA, 2021b)
- 10,220,000,000 g over 450,000,000,000 m^2 = 0.227111 g m^{-2}
- Enteric emissions in gigagrams from the bovine and ovine livestock of the UK
- 6,156,000 head of cattle and 19,144,000 sheep in 2000 (DEFRA, 2010)
- Cattle emit 12,610,000,000 g and sheep 3.680,000,000 g
- Livestock emissions / population = grams per head
- Average British cow weight = 600 kg
- Average British sheep weight is more variable, common breeds are Texel (44 kg), Suffolk (88 kg), Charollais (100 kg) so taking an approximation of 80 kg
- Dividing weight per head by average kg = emissions kg $liveweight^{-1}$

Stewart et al (2014) also gave enteric emissions by area and not liveweight, carcass or head.

- LSU values given in the study produced a per head value over the given area
- The study also gave livestock end weight means, allowing a division to kg $liveweight^{-1}$

Thomas (2012) gave livestock emissions by carcass. A carcass usually weighs 2/3 the liveweight of the animal

- Emissions were increased by 1/3 to account for liveweight
- Then divided by cattle weight for a kg $liveweight^{-1}$ value

Other conversions

CO_2-eq to CH_4 = /25

CO_2-eq to N_2O = /298

C to CO_2: (12+16+16)/12 = CO_2 total mass using molecular weight, calculate CO_2 from C:CO_2 ratio

N to N_2O: (14+14+16)/(14+14) = N_2O total mass using molecular weight, for N:N_2O ratio

C to CH_4: (12+1+1+1+1)/12 = CH_4 total mass using molecular weight, for C:CH_4 ratio

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