Impacts of climate change and land management on carbon dynamics of British upland grassland soils

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 2

Eze, S., Palmer, S.M. & Chapman, P.J. *In Review* in Journal of Environmental Management. Soil organic carbon stock in grasslands: effects of inorganic fertilizers, liming and grazing in different climate settings.

SE (the candidate) gathered peer-reviewed articles, screened the articles, extracted data, analyzed the data and wrote the manuscript. All authors set the criteria used in selecting and screening peer-reviewed articles used for data extraction. All authors examined the database prepared after data extraction and decided on the set of data to be included in the study. SMP and PJC (PhD supervisors) reviewed all drafts of the manuscript and gave critical feedback, advice and guidance.

Chapter 3

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SE designed the study, conducted laboratory analysis, performed statistical analysis and wrote the manuscript. All authors were involved in planning the study, identifying study sites and collecting soil samples. SMP and PJC reviewed all drafts of the manuscript and gave critical feedback, advice and guidance.

Chapter 4

Eze, S., Palmer, S.M. & Chapman, P.J. 2018. Upland grasslands in northern England were atmospheric carbon sinks regardless of management regimes. *Agricultural and Forest Meteorology,* 256 – 257, 231 – 241.

SE designed the study, built soil collars, conducted field and laboratory gas flux measurements, performed statistical analysis and wrote the manuscript. All authors were involved in planning the study, identifying study sites, fixing collars in the soil prior to gas measurements, and removing flux equipment from the field. SMP and PJC accompanied SE to the sites from time to time and assisted with gas sampling. SMP and PJC reviewed all drafts of the manuscript and gave critical feedback, advice and guidance.

Chapter 5

Eze, S., Palmer, S.M. & Chapman, P.J. 2018. Negative effects of climate change on upland grassland productivity and carbon fluxes are not attenuated by nitrogen status. *Science of the Total Environment*, 637 – 638C, 398 – 407.

SE designed the experiment, built environmental chambers and installed all equipment used in the chambers, built lysimeters, constructed soil collars and its covers, set the environmental room and chamber conditions, measured carbon fluxes, conducted laboratory analysis, performed statistical analysis and wrote the manuscript. SMP and PJC reviewed the initial experimental design and identified aspects of the design that needed changes. SMP and PJC reviewed all drafts of the manuscript and gave critical feedback, advice and guidance.

Rationale for thesis by alternative format

This thesis is submitted as an alternative style of doctoral thesis including published material. This format is appropriate for this thesis because three out of the four data chapters have been published in peer-reviewed journals. The fourth data chapter is currently in review. Loose copies of the published manuscripts accompany this thesis. The thesis is divided into six chapters, which is in line with the Faculty of Environment protocol for presenting an alternative style of doctoral thesis including published material. Chapter 1 contains an overview of available literature which gave context to the thesis and established its relevance, research gaps and rationale for the study, research questions and objectives, and an outline of research approach. Chapters 2 - 5 are the four manuscripts. Chapter 6 is the synthesis of the main findings in the thesis. It draws together the findings in Chapters 2 – 5, and discusses the wider implications of the findings. Chapter 6 also contains the limitations of the study, directions for future work, and a summary of conclusions from the thesis.

Assertion of moral rights:

The right of Samuel Eze to be identified as Author of this work has been asserted by him in accordance with the Copyright, Designs and Patents Act 1988.

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Abstract

This thesis sought to improve understanding of the effects of typical management regimes (conventional pasture and traditional hay meadow under agri-environment schemes) in the British upland grasslands and climate change on soil carbon (C) dynamics. Two upland grassland locations in northern England, UK were investigated. Results showed that the upland grasslands were a net atmospheric C sink (1822 – 2758 g CO_2-eq m^{-2} $year^{-1}$), with a significant SOC stock (59 – 101 Mg ha^{-1}) which was predominantly (60%) protected in the soil mineral mass. The conventional pasture site receiving inorganic nitrogen (N) addition had a significantly higher SOC stock (101 Mg ha^{-1}) and the greatest ecosystem C uptake (2758 g CO_2-eq m^{-2} $year^{-1}$) compared to all other sites. Experimental summer climate of late 21st Century (+3°C increase in temperature and -23% decrease in precipitation) resulted in a significant decline in the net ecosystem C sink (-13 to -29%) and plant biomass productivity (-29 to -77%) of the grasslands, regardless of management regimes. Overall, the results indicate that both conventional pasture and hay meadow management regimes maintain C sequestration in the upland grasslands, with the hay meadow management being less efficient. Also, warmer climate and drought by the end of the 21st Century will most likely lead to a significant decline in both C sink and forage production in the upland grasslands regardless of management. Moderate levels of inorganic N fertilizers plus organic manures is recommended to enhance biomass production and C sequestration in the traditional hay meadows. In addition, hay mulching is recommended as a viable management option to mitigate against the potential negative effects of climate change on both biomass productivity and C sequestration. Finally, pilot studies on a greater number of managed grasslands are needed to test the effectiveness of the recommended management changes for providing targeted outcomes.

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Abbreviations

AGB – aboveground biomass

Al - Aluminium

BGB – belowground biomass

BREXIT – Britain’s decision to leave the European Union

C - Carbon

Ca – Calcium

$CaCO_3$ – Calcium carbonate

CAP – Common Agricultural Policy

CC – cylindrical chamber

CH_4 – Methane

CO_2 – Carbon dioxide

CO_2-eq – carbon dioxide equivalent emissions

CV – coefficient of variation

DAGB – dead aboveground biomass

DEFRA – Department for Environment, Food and Rural Affairs

DOC – dissolved organic carbon

EC – environmental chamber

ER – ecosystem respiration

FU – forage utilization

FYM – farmyard manure

GC – Gas chromatograph

GHG – greenhouse gas

GPP – gross primary productivity

GWP – global warming potential

HNF – high nitrogen field

IPCC – Intergovernmental Panel on Climate Change

K – Potassium

KCl – Potassium chloride

LAGB – live aboveground biomass

LNF – low nitrogen field

MAP – mean annual precipitation

MAT – mean annual temperature

MBC – microbial biomass carbon

Mg – Magnesium

N – Nitrogen

Na – Sodium

NECB – net ecosystem carbon balance

NEE – net ecosystem exchange

NO_3 - Nitrate

NPP – net primary productivity

N_2O – Nitrous oxide

OM – organic matter

P – Phosphorus

P_{+15} - ambient precipitation plus 15%

P_0 – ambient precipitation

P_{-23} – ambient precipitation minus 23%

PAR – photosynthetically active radiation

Pg - Petagram

POC – particulate organic carbon

POM – Particulate organic matter

Q_B – between group heterogeneity

Q_T – total heterogeneity

Q_W – within group heterogeneity

rSOC – chemically resistant soil organic carbon

S+A – sand and stable soil aggregates

s+c – silt and clay

SIC – soil inorganic carbon

SOC – soil organic carbon

SOM – soil organic matter

SR – Soil respiration

T_0 – ambient air temperature

T_3 – ambient air temperature plus 3°C

TAGB – total aboveground biomass

UK – United Kingdom

Chapter 1: Introduction

Soils contain a large amount of organic carbon (e.g. 1417 Pg at 1 m depth globally; 1 Pg = 10^{15} g; Hiederer and Kochy, 2011), which is about three times the amount in vegetation and twice the amount in the atmosphere (Lal, 2013). Changes in soil carbon (C) stock lead to either an increase or a decrease in atmospheric carbon dioxide (CO_2) and methane (CH_4) concentrations, which has implication for global warming and climate change. World soils have lost an estimated 79 Pg of its organic C due to agricultural intensification and soil degradation (Lal, 1999). However, this soil organic C (SOC) loss can be reversed through sequestration, which is the removal of atmospheric C and its storage in stable forms in the soil. Soils have the potential to sequester 1.2 – 3.1 Pg C year^{-1} globally, which is equivalent to the removal of over 0.47 ppm of atmospheric CO_2 annually (Lal, 2003). SOC sequestration is therefore considered the most cost-effective solution to climate change mitigation (Lal, 2013) as other technologies for removing atmospheric C are not yet viable. SOC sequestration also has additional benefits of improving soil health, food security and environmental quality (Lal, 2016).

The need to reduce C emissions and the importance of SOC sequestration in this regard was recognized during climate change negotiations at the 21st Conference of the Parties to the United Nations Framework Convention on Climate Change in Paris in 2015 (COP21). At this conference, an action plan “4 per mille Soils for Food Security and Climate” was proposed to increase global SOC stock by 0.4% annually, through appropriate management practices such as conservation agriculture (Minasny *et al.,* 2017). However, lack of sufficient evidence on the effectiveness of recommended management practices and the paucity of data on the C sink capacity of many soils, make it difficult for governments to implement the “4 per mille” initiative (Lal, 2016).

Managed agricultural soils have the greatest potential for sequestering atmospheric C but this varies in response to climate, aboveground vegetation type, management practices and soil characteristics (Minasny *et al.*, 2017; Olsson *et al.,* 2009). Therefore, it is important that the most appropriate agricultural soils, for example those with depleted SOC due to past management practices and those with soil properties that favour SOC retention in stable forms, are targeted for C sequestration. An improved understanding of organic C dynamics in soils of specific eco-regions that are subjected to different management practices would help to identify management activities that are suitable for enhancing SOC storage and stability.

1.1 Carbon storage and its dynamics in grassland soils

Grassland soils contain about 20% of the world's total SOC (Jobbágy and Jackson, 2000; Table 1.1), up to 32% of the total SOC in the UK (Ostle *et al.,* 2009), and often have higher C stock than other vegetation types in a given climate regime (IPCC, 2003; Yang *et al.,* 2010). This is possible because approximately 90% of atmospheric C sequestered by grasslands is stored in the soil, due to high root biomass and a rapid annual turnover of shoot materials (Reeder and Schuman, 2002). Grasslands are therefore considered an important global C store that can function as either a C sink or source (Heinemeyer *et al.,* 2012) depending on management and site characteristics such as climate, and this has implication for either mitigating or reinforcing climate change.

Table 1.1: Carbon stock in grassland soils to 1 m depth.

Grassland soils	Land area (10^{12} m^2)	Soil organic carbon stock (Pg C)	Reference
Tropical grasslands/savannah	15.00	198	Jobbágy and Jackson, 2000
Temperate grasslands	9.00	105	Jobbágy and Jackson, 2000
British grasslands	0.08	2	Ward *et al.,* 2016

1 Pg = 10^{15} g.

C dynamics in terrestrial ecosystems involve plant uptake of atmospheric C via photosynthesis and its release back into the atmosphere through plant and microbial respiration, or into groundwater or surface waters via leaching and erosion (Figure 1.1). Soils also take up atmospheric C directly when CH_4 is oxidised by methanotrophs under aerobic conditions and release it into the atmosphere when soil organic matter (SOM) is decomposed under anaerobic conditions (Van den Pol-van Dasselaar *et al.,* 1999). The net balance between these processes of C uptake and C loss determines an ecosystem's net C sequestration and potential contribution to climate change mitigation. An important C flux measure that has been used to assess the C sink-source status of terrestrial ecosystems is net ecosystem exchange (NEE), which is the difference between CO_2 uptake via photosynthesis (gross primary productivity, GPP) and CO_2 released by respiration (ecosystem respiration, ER) (Chen *et al.,* 2013; Kang *et al.,* 2013). NEE reflects the net C accumulation by both plants and soils.

Grasslands' NEE varies greatly, with both negative (C sink) and positive (C source) values reported in different parts of the globe (see Novick *et al.*, 2004). This variability has been attributed to differences in management activities and specific site characteristics such as soil type, soil temperature and moisture (Jacobs *et al.,* 2007; Polley *et al.*, 2008). The responses of NEE to grassland management activities such as grazing (Haferkamp and MacNeil, 2004; Kjelgaard *et al.,* 2008; Owensby *et al.*, 2006; Skinner, 2008) and fertilizer application (Bardgett and Wardle, 2003; Fang *et al.,* 2012; Harpole *et al.*, 2007; Welker *et al*., 2004; Xia *et al.,* 2009) are not consistent, with both negative and positive effects reported. This may be due to the strong influence of climate and soil characteristics on C cycling processes. Climate influences all C cycling processes including plant growth and microbial activities (De Deyn *et al.,* 2008), whereas retention belowground, protection from microbial decomposition and stability of soil C are strongly influenced by soil characteristics (Dungait *et al.,* 2012). More details of the effects of climatic zone and soil characteristics on C dynamics are presented in the following sections.

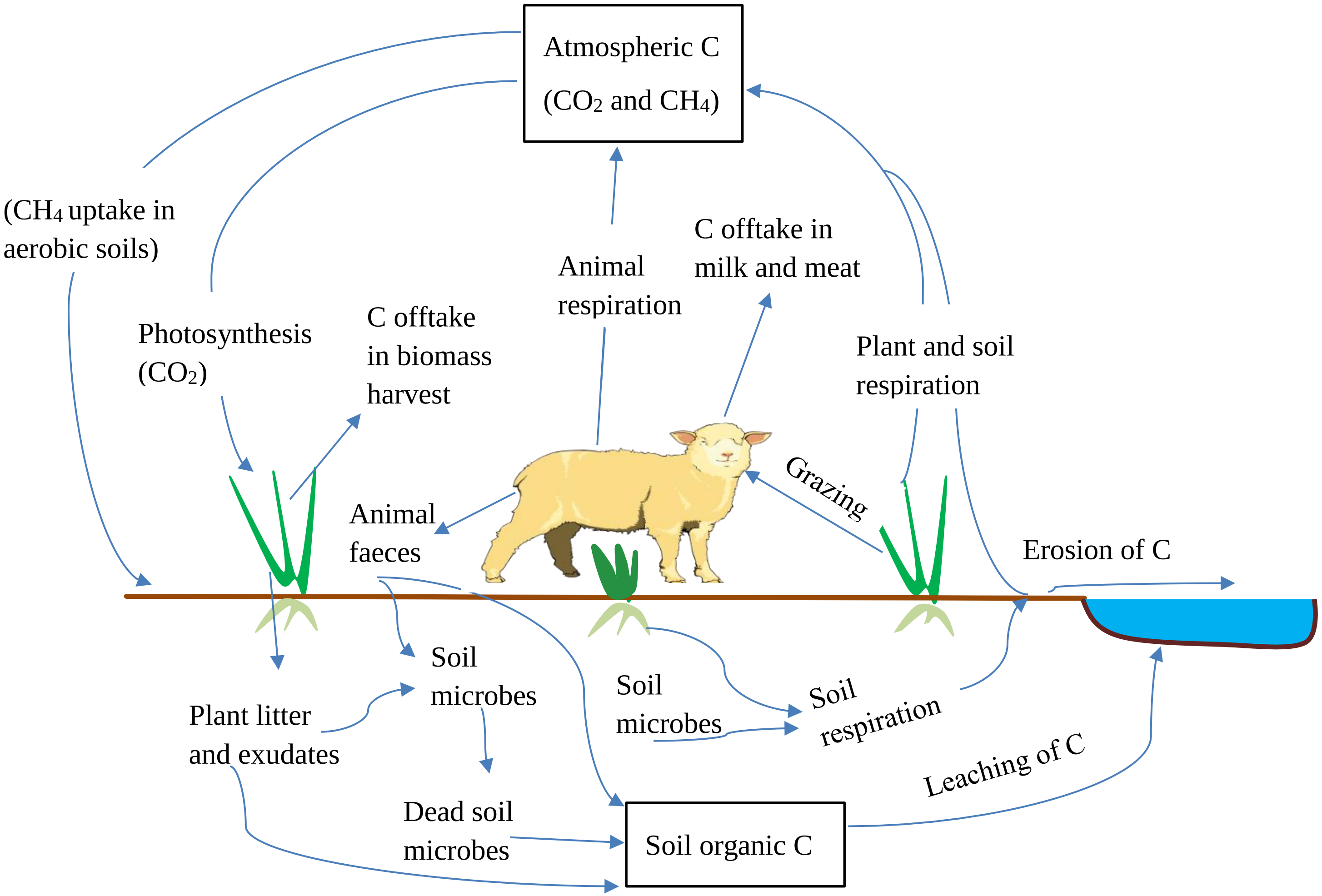


Figure 1.1: Carbon (C) dynamics in grasslands.

1.1.1 Influence of climatic zone on C dynamics

The dynamics of C in an ecosystem is a function of the prevailing climate. Climate influences the distribution of vegetation on the landscape (White *et al.,* 2000), the uptake of atmospheric C by plants, and the turnover of the C stored in both plants and soils (Carvalhais *et al.*, 2014). Hence, climate influences the distribution of SOC stock globally (Table 1.2), with greater SOC stock in the cool and humid climatic zones than in the warmer and drier areas (Hobbie *et al.,* 2000).

Table 1.2: Distribution of soil organic carbon stock in different climatic zones (Scharlemann *et al.,* 2014).

Climate	Soil organic carbon stock in 1 m depth (Pg)
Tropical wet	128
Tropical moist	151
Tropical dry	136
Tropical montane	56
Warm temperate moist	63
Warm temperate dry	79
Cool temperate moist	210
Cool temperate dry	102
Boreal moist	357
Boreal dry	69
Polar moist	52
Polar dry	12
Total	1415

1 Pg = 10^{15} g.

The prevailing wet conditions under cool and humid climate restricts drainage and coupled with low temperatures, contributes to slow decomposition of organic materials and the accumulation of organic C. Sometimes, however, plant productivity may be restricted under cold conditions thereby reducing C inputs to soil and SOC stock (Garcia-Pausas *et al.,* 2007). Overall, net uptake of atmospheric C (negative NEE) in managed grassland ecosystems has been reported in areas where soil moisture was not limiting for plant growth (Hao *et al.,* 2013; Rigge *et al.,* 2013). On the other hand, net C efflux (positive NEE) in managed grasslands has been associated with seasonal increase in soil temperature (Cui *et al.,* 2014; Frank *et al.,* 2002; Jones *et al.,* 2006), and when soil moisture available for plant growth is reduced during periods of high evapotranspiration and low precipitation (Novick *et al.,* 2004; Wang *et al.,* 2016).

Although climate seems to play a prime role in terrestrial C cycling, the influence of management activities on C balance in grasslands is still significant (Chang *et al.,* 2015). A number of studies have investigated the separate effects of grassland management activities and climatic variables on soil C (see Sections 1.2.4 and 1.3.3). However, the effects of management practices on SOC in different climatic settings have not been synthesized on a global scale. An improved understanding of how SOC in different climate zones respond to management practices will help in devising management strategies that are appropriate for enhancing C sequestration in each grassland region of the globe.

1.1.2 Influence of soil characteristics on C dynamics

Soil characteristics such as texture, structure, moisture content and pH influence plant growth (i.e. accumulation of C in plants), microbial decomposition of organic materials and SOC storage. For example, soil acidification ($pH < 4.5$) has been found to reduce plant growth (Soti *et al.,* 2015), and suppress soil C efflux in fertilized grasslands (Chen *et al.,* 2016). Also, soil texture determines soil porosity, drainage, and the availability of water and nutrient to plants (Grellier *et al.,* 2014). When soil water and nutrients are available and accessible to plants under suitable temperature conditions, there is an increase in plant growth (Cristiano *et al.,* 2015; Seagle and McNaughton, 1993), which leads to greater return of organic C to the soil. The fate of the organic C in soils is also dependent on soil texture, particularly the clay content. Clay minerals offer physical protection to SOC via surface adsorption (Singh *et al.,* 2016). This physical protection of SOC from microbial decomposition increases SOC stock (Mao *et al.,* 2015; Yang *et al.,* 2008), and ensures that SOC is stabilized in long term pools that are less susceptible to loss (Adkins *et al.,* 2016). The stabilization of SOC in long term pools is an area of current research interest because of its relevance to climate change mitigation.

To assess the relative stability of SOC, it is usually fractionated into conceptually functional pools such as labile (or active) and stable (or passive) pools, with different turnover rates or mean residence times, ranging from days to centuries and millennia (Breulmann *et al.,* 2016; Von Lützow *et al.,* 2007). The active C pools such as particulate organic C (POC), dissolved organic C (DOC) and microbial biomass C (MBC) have the fastest turnover and are more sensitive to land management and environmental change than the stable pools (Alvarez and Alvarez, 2016; De Figueiredo *et al.,* 2010; Van Leeuwen *et al.,* 2015). Grazing has been shown to reduce the labile soil C pools (Wen *et al.,* 2016; Wiesmeier *et al.,* 2012). However, the effects of other management activities such as N fertilization on C pools across different grasslands are inconsistent due to site specific characteristics (Riggs *et al.,* 2015). Thus, there is need to assess not only the fractional distribution of SOC stock in managed grasslands, but also to account for both management differences and site characteristics such as soil type and soil parent material. This will help to identify management activities that are appropriate for enhancing soil C stabilization under different site characteristics.

Soil parent material needs to be considered in SOC studies because it influences soil physical and chemical properties that are important for C flux (Shrestha *et al.,* 2014) and SOC storage. For example, acid-forming parent materials such as siliceous stones and alkaline-forming parent materials such as limestone exert significant control on the pH of their resulting soils (Mijangos *et al.,* 2010), and soil pH correlates positively with soil CO_2 efflux (Chen *et al.,* 2016). Soil parent material also exerts a strong influence on soil texture and mineralogy (Araujo *et al.,* 2017), which determine the surface area of soil particles available for organic C occlusion (a form of physical protection) as well as soil moisture retention and availability to both plants and soil microbes. Thus, the choice of appropriate grassland management for enhancing C storage requires an improved understanding of the dynamics of C in soils developed on different parent materials.

This section shows that grassland soils are important for C storage. However, the influence of soil characteristics, climate and management activities on the dynamics of C in grassland soils is not well understood. In the next section, what is currently known about the effects of typical grassland management activities on SOC is discussed.

1.2 Grassland management and soil carbon storage

Globally, grasslands are managed to increase biomass productivity in order to support livestock production. They are therefore being directly grazed, cut for fodder typically as hay or silage, or a combination of all three. Management activities are primarily used to change the status of soil properties thereby creating optimum conditions for plant growth. For example, nutrient levels and pH status can be improved by fertilizer application and liming respectively, and these are typically the most common management activities for improving or maintaining grassland productivity. As well as the intended increase in aboveground biomass, fertilisation and liming potentially lead to greater production of root exudates and litter, and often have unintended effects on soil properties such as microbial populations and their activities that influence decomposition processes (Alonso *et al.,* 2012; Hoffman *et al.,* 2014; Soussana *et al.,* 2007). These management activities therefore have implications for soil C storage and sequestration, which are discussed in the following sections.

1.2.1 Fertilizer application and soil carbon storage

Fertilizers are often applied to supply the soil with one or more nutrients such as nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg). However, there is evidence that N-only fertilizers have greater effects on SOC storage than multi-nutrient fertilizers which combines N with other nutrients (Fornara *et al.,* 2013). Also, most studies investigating SOC response to fertilizer application have focused on N addition (e.g. He *et al.,* 2013; Lu *et al.,* 2011; Yue *et al.,* 2016). Although most of these studies found that N fertilizer application led to an increase in SOC stock, some have reported the opposite effect. An understanding of the mechanisms of interaction (Figure 1.2) between added N and ecosystem processes would help explain why these contradictory effects have been observed.

N-induced increase in soil C stock could result from a number of processes. Firstly, N fertilizers have been found to increase plants' net primary productivity, which may translate into higher biomass inputs below ground and enhanced soil C storage (Ward *et al.,* 2016; Yue *et al.,* 2016). Apart from stimulating aboveground productivity, in nutrient-limited grasslands, N additions may also enhance plant root production leading to increased soil C (Ziter and MacDougall, 2013). Also, based on the study of an upland grassland in Wales, Bardgett *et al.* (1999) demonstrated that N additions increased rhizosphere acidity which significantly changed the soil microbial community structure in favour of fungi. As fungal decomposition of organic materials occurs at a slower rate than other microbes such as bacteria (Badgett *et al.,* 1993), N-induced increase in soil acidity and the dominance of fungi would most likely result in soil C accumulation. When other nutrients such as P are limited, the effects of N addition on soil microbial community may be intensified (Ziter and MacDougall, 2013), resulting in a significant reduction in the microbial decomposition of organic materials and CO_2 efflux from the soil (Treseder, 2008).

A number of processes have also been put forward to explain the reduction in soil C storage attributed to fertilizer application in some grasslands. Firstly, plant species richness and diversity which have been associated with enhanced SOC storage (De Deyn *et al.,* 2011) could be significantly reduced by the addition of fertilizers. Fertilizer application increases competition among plant species, resulting in the reduction of species richness in grasslands (Silvertown *et al.,* 2006; Smith *et al.,* 1996; Socher *et al.,* 2012). For example, a widespread loss of species-rich grasslands in mainland Europe and particularly Britain has been attributed to the increased use of fertilizers (Kirkham *et al.,* 2014). The decrease in plant species' diversity and richness may lead to reduced net C storage in soils (Lange *et al.,* 2015). Increased N availability has also been shown to alter C allocation by plants, favouring C accumulation in the aboveground parts and lower inputs to below ground parts (Johnson and Thornley, 1987; Ziter and MacDougall, 2013). However, this may not always result in soil C loss because the ultimate fate of the aboveground vegetation will depend on whether it is being removed from the field via grazing, hay/silage or returns as litter to the soil. There is also a positive response of DOC to N availability (Pregitzer *et al.,* 2004), and this further suggests that increasing soil N through fertilization may result in the net loss of C. Finally, N-induced decrease in soil C storage may result from increased decomposition of plant litter due to its enhanced quality (Yue *et al.,* 2016). Berg and Laskowski (2006) have linked increase in plant litter quality (reduction in C/N ratio or lignin/N ratio) to N additions, which increases the inputs to soil of labile C that is more susceptible to microbial decomposition.

In reality, all the processes described in this section and presented in Figure 1.2 occur together and whether N addition results in an increase or a decrease in SOC stock depends on how the processes interact. The net effect of N addition on SOC will also depend on the interactive effects of other management activities such as liming and grazing regime.

For example, grazing intensity and livestock stocking density will determine the amount of vegetation that is removed from a grazed grassland. This will in turn affect the amount of litter returned to the soil and thus SOC stock.

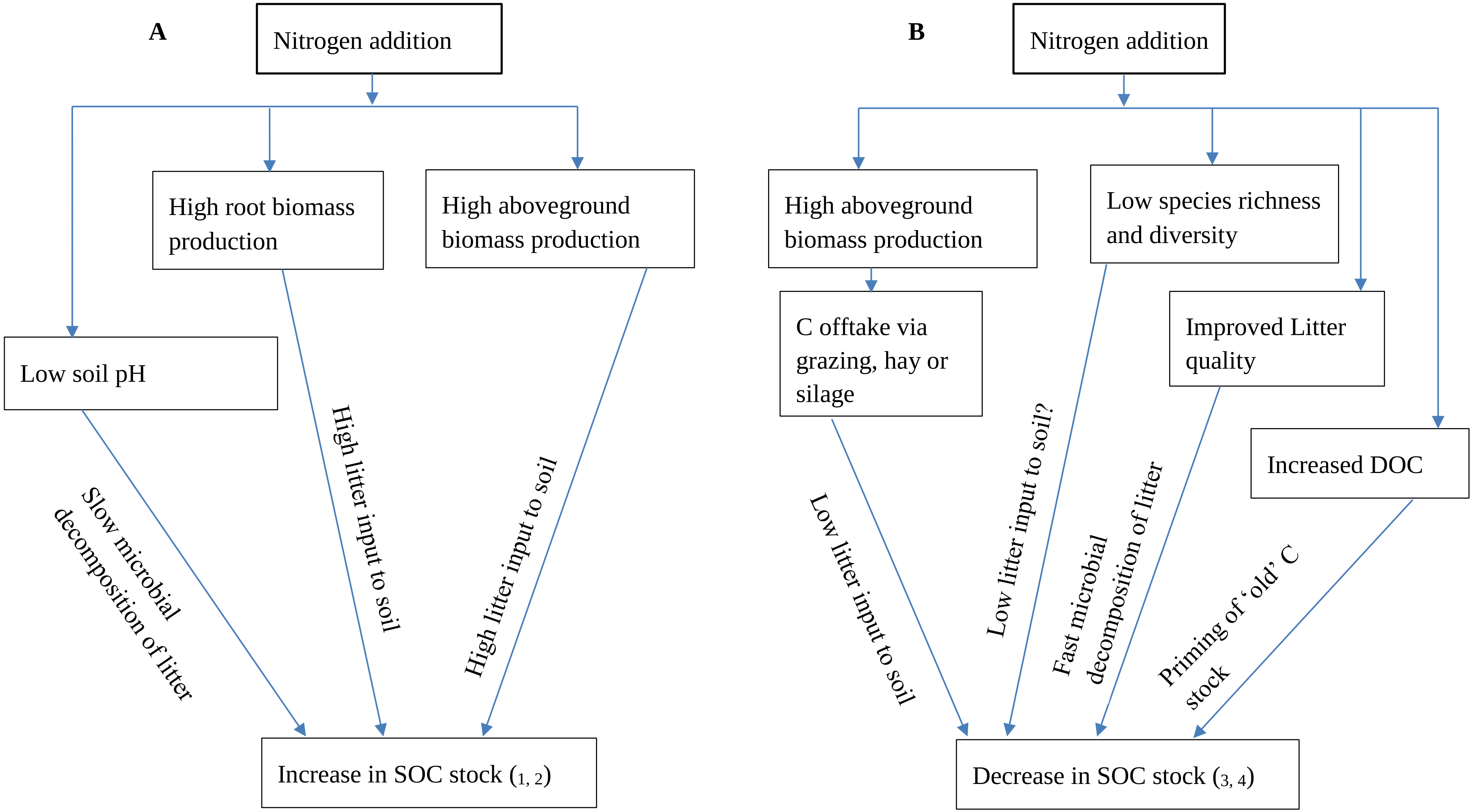


Figure 1.2: Mechanisms through which nitrogen fertilizer application enhances (A) or depletes (B) soil organic carbon (SOC) stock. DOC = dissolved organic carbon. 1 = Ziter and MacDougall, 2013; 2 = Yue *et al.*, 2016; 3 = Pregitzer *et al.*, 2004; 4 = Lange *et al.*, 2015.

1.2.2 Liming and soil carbon storage

The pathways of C in the soil when lime is applied can be grouped into three broad categories: the dissolution pathway, the biological productivity pathway and the structural protection pathway (Figure 1.3). The dissolution pathway of soil C dynamics describes what happens to soil C as liming materials such as limestone ($CaCO_3$) and dolomite ($CaCO_3MgCO_3$) are dissolved in soil solution. When these liming materials are applied to the soil, any of the following reactions takes place (Hamilton *et al.,* 2007; West and McBride, 2005):

$$CaCO_3 + H_2O + CO_2 \rightarrow Ca^{2+} + 2HCO_3^{-} \tag{1.1}$$

$$MgCa(CO_3)_2 + 2H_2O + 2CO_2 \rightarrow Mg^{2+} + Ca^{2+} + 4HCO_3^{-} \tag{1.2}$$

$$CaCO_3 + 2HNO_3 \rightarrow Ca^{2+} + 2NO_3^{-} + H_2O + CO_2 \tag{1.3}$$

$$MgCa(CO_3)_2 + 4HNO_3 \rightarrow Ca^{2+} + 4NO_3^{-} + 2H_2O + 2CO_2 \tag{1.4}$$

The reaction that takes place depends on the type of liming material (whether limestone or dolomite) as well as the pH of the soil solution. According to Hamilton *et al.* (2007), when the soil is moderately acidic (pH 5.0), almost all lime dissolution is caused by weak acids particularly carbonic acid and the first two reactions (equations 1 and 2) dominate. The carbonic acid is mostly produced by the dissolution in water of CO_2 from both root and microbial respiration. When equation 1.1 or 1.2 dominates, liming results mostly in the soil acting as a C sink because for every mole of lime-derived C that dissolves, one mole of CO_2 from the soil is converted to bicarbonate. This bicarbonate may either remain in the soil or get leached through the soil profile into surface and/or ground waters and finally to the ocean where it precipitates as $CaCO_3$ (West and McBride, 2005). At lower soil pH and in the presence of a strong acid such as HNO_3 mostly produced by the nitrification of N fertilizers, the dissolution of lime materials acts as a CO_2 source rather than a sink because for every mole of lime-derived C, 1 mole of CO_2 is released (Hamilton *et al.,* 2007). Equations 1.1 – 1.4 therefore suggest that liming of an acidic soil may initially result in lime-derived C loss from the soil but when the pH is 5.0 and above, and in the absence of N fertilizers, additional liming may lead to the soil acting as a net inorganic C sink.

The second pathway of soil C dynamics under liming is biological productivity. This concerns the growth-stimulating effect of liming on both soil organisms and plants. As liming raises pH to optimal levels for soil biota, increases nutrient availability, and reduces micronutrient toxicity, the soil microbial activities as well as plant productivity are likely to increase significantly (Li *et al.,* 2014; Pabian *et al.,* 2012). This has two possible effects on net soil C stock (Paradelo *et al.,* 2015). Firstly, when liming induces an increase in plant growth and productivity, organic matter (OM) returns to the soil in the form of litter, decaying roots, root exudates, and sloughed root cells will be high; this may consequently lead to higher SOC storage. On the other hand, increased activity of soil biota due to liming is likely to increase SOC loss through enhanced decomposition of organic materials.

Finally, the structural protection pathway describes the processes of lime-induced aggregation of soil particles into stable structures and subsequent encapsulation of SOC within the soil aggregates. Liming influences soil structural aggregate formation through dispersion or flocculation. Haynes and Naidu (1998) explained that at low pH, acid soils are normally flocculated because of the attraction between Al^{3+}, Fe^{3+} and H^{+} and the negatively charged clay surfaces, as well as the adsorption of OM. As liming raises pH, the polyvalent cations precipitate leaving a net negative charge on the soil surfaces which results in the dominance of repulsive forces that disperses the soil particles. However, soil structure can be improved due to the cementing actions of liming materials such as $CaCO_3$; also, at high pH (> 6.0) the high concentration of Ca^{2+} results in the decrease of repulsive force between soil particles thereby increasing aggregation of soil particles. The precipitated Al in the form of hydroxy-Al polymers may also serve as cementing agents in improving soil structures.

The three pathways of soil C dynamics under liming operate interdependently. For instance, it was shown that the dissolution of lime in a low pH condition results in CO_2 emission but when present in sufficient quantity $CaCO_3$ in soil solution may act as a binding agent thereby stabilizing soil aggregates and promoting C storage. Also, lime-induced increase in mineralisation is known to increase respiratory C loss but increased microbial activity can increase C storage through enhanced aggregate stabilization. Microbial-mediated aggregation of soil particles occurs because soil microbes produce binding agents in the form of extracellular gelatinous polysaccharides and soil fungi can form a network of enmeshing hyphae (Haynes and Naidu, 1998; Paradelo *et al.,* 2015). In view of these complex interactions, the net effect of liming on soil C is likely to vary temporally (due to seasonal changes in climate, litter inputs and decomposition rates) and spatially due to differences in site-specific characteristics such as initial soil pH and the soil’s buffering capacity. The buffering capacity of the soil refers to its ability to resist change in pH and depends on the amount of clay and OM, which possess charged surfaces for the adsorption of H^+.

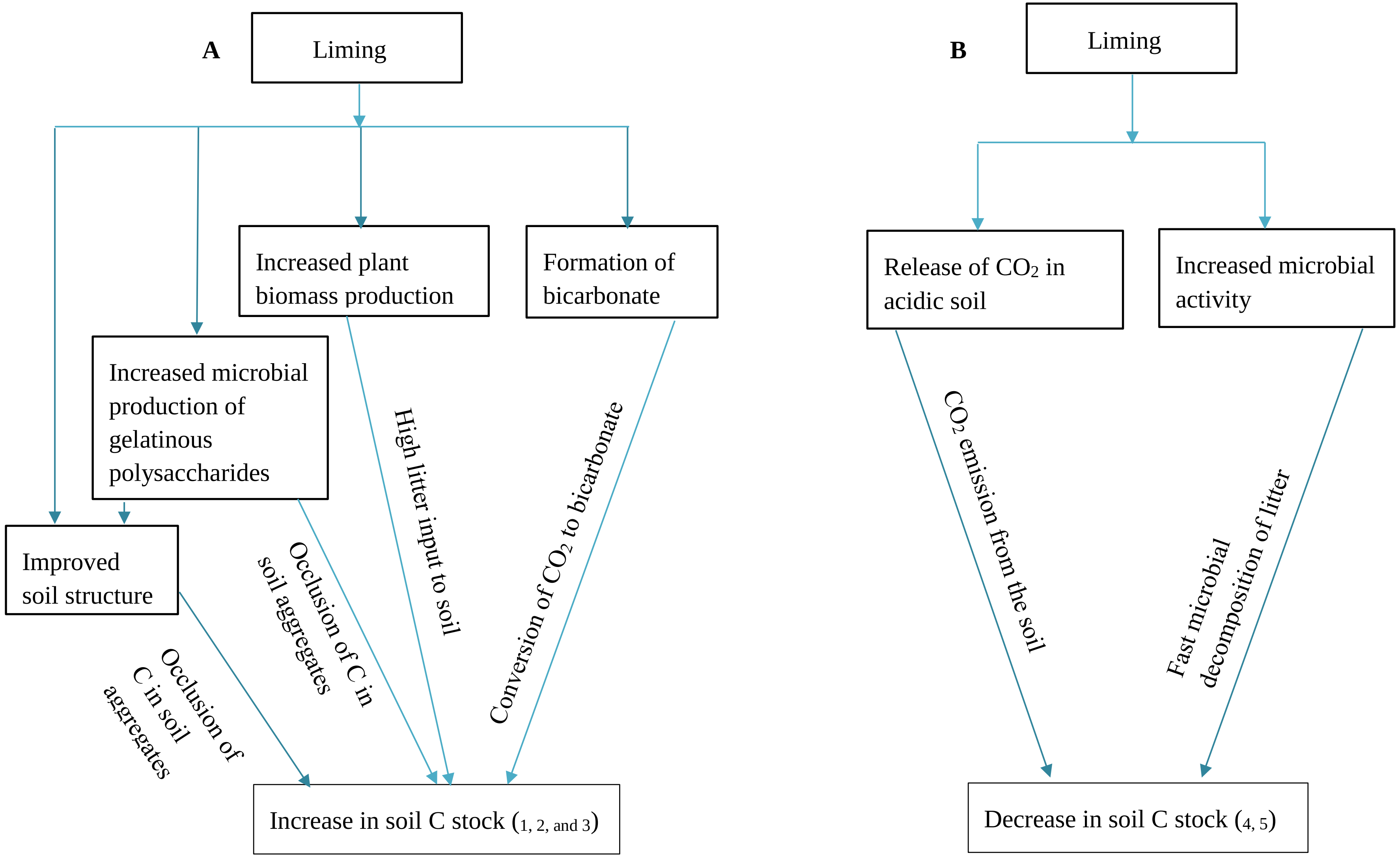


Figure 1.3: Mechanisms through which liming enhances (A) or depletes (B) soil carbon (C) stock. 1 = Haynes and Naidu, 1998; 2 = West and McBride, 2005; 3 = Paradelo *et al.*, 2015; 4 = Hamilton *et al.*, 2007; 5 = Paradelo *et al.*, 2015.

1.2.3 Grazing regime and soil carbon storage

Besides fertilizer application and liming, grazing regime also influences net soil C storage (Figure 1.4), but the exact effect depends on the intensity of grazing. Grazing intensity is often grouped into light/low density grazing, moderate grazing and heavy grazing, based on the number of livestock used for grazing, the frequency of grazing, or the amount of vegetation removed by grazing animals. For example, less than 40%, 40 – 65%, and more than 65% vegetation utilization by grazing animals are considered light, moderate, and heavy grazing, respectively (Evans *et al*., 2012; Krzic *et al.*, 2014). A low density sheep grazing (e.g. less than 2 sheep ha^{-1} or 35% vegetation utilization) has been reported to cause no significant change in SOC stock relative to ungrazed sites (Reeder *et al.*, 2004; Ward *et al.*, 2007) because C losses were balanced by C gains. As the grazing intensity increased (e.g. 5 sheep ha^{-1} or 40% utilization of vegetation), soil C stock increased (e.g. Chen *et al.*, 2015). This grazing-induced increase in soil C stock could result from over-compensatory plant growth following defoliation (Conant and Paustian, 2002) as well as increase in inputs from enhanced root production (Frank *et al.*, 2002). Over-compensatory growth after grazing indicates a regrowth of vegetation with greater biomass than initially removed by the grazing livestock (Tanentzap and Coomes, 2012). This is possible because most grassland species have their meristems at the base of the plants which allow for rapid regrowth following the removal of the aboveground parts (Tanentzap and Coomes, 2012). The grazing threshold that favours the process of over-compensation is still unknown and it is debated whether there is community level regrowth that equals or is greater than pre-defoliation biomass levels (Tanentzap and Coomes, 2012).

Grazing could also lead to a net soil C loss for a number of reasons. Firstly, excessive removal of vegetation from grasslands during grazing, which is made worse when the grasslands are mowed for hay or silage, results in soil C loss (Mestdagh *et al.*, 2006). This happens because the C removed in aboveground litter is not adequately compensated for by livestock excreta or belowground production, and livestock are removed from the system for meat or when they die. For example, Soussana *et al.* (2007) observed that a large proportion of organic C ingested by grazing livestock is lost through respiration, enteric fermentation and export of products such as milk, with only about 25-40% of ingested C returned to the grassland as excreta. Grazing could also result in the exhaustion of root reserves (Mestdagh *et al.*, 2006), reduction in plant growth capacity (Conant and Paustian, 2002), and the overall degradation of grasslands characterised by low productivity (Su *et al.,* 2015). As net primary production decreases due to degradation, C inputs to the soil will also decrease resulting in soil C decline.

Livestock grazing also influences the abundance and diversity of soil organisms which affects the rate of OM decomposition and C dynamics (Bardgett *et al.,* 1993). Medina-Roldán *et al.* (2012) found that 7 years of grazing exclusion resulted in a 20% reduction in the activity of soil microorganisms. This may be due to the absence of animal excreta which contains readily utilizable substrates that stimulate soil microbial activities in grasslands (Clegg, 2006). Williams *et al.* (2000) reported an increase in bacteria number relative to fungi with addition of synthetic sheep urine, a relatively available N source. Ritz *et al.* (2004) also found a high microbial biomass within the soil due to increasing sheep urine patches. It is therefore plausible to think that sheep grazing, which has been shown to increase bacteria community and microbial activity in grasslands (Grayston *et al.,* 2004), would result in the loss of SOC. This is because sites with bacterial dominance relative to fungi are associated with greater OM decomposition and lower soil C levels (Chen *et al.,* 2015). However, increasing microbial activity may not always result in soil C loss as mineralisation by the microbes may release nutrients that promote vegetation growth and greater litter inputs.

Grazing may also cause soil C loss through trampling and poaching. Trampling from grazing animals results in soil compaction, characterised by increased bulk density and reduced infiltration (Marshall *et al.,* 2009; Marshall *et al.,* 2014). This increases overland flow during storm events, and can lead to soil erosion and the loss of particulate soil C (Meyles *et al.,* 2006). Simulated sheep trampling has also resulted in a significant decrease in the rate of photosynthesis (Clay and Worrall, 2013), which may lead to lower inputs of C to the soil. However, livestock trampling during grazing may not always lead to soil C loss because an increase in the bulk density of mineral soils reduces soil CO_2 efflux (Pengthamkeerati *et al.,* 2005) and DOC loss (Beare *et al.,* 2009). As the bulk density increases, soil pores close up and this minimizes the release of CO_2 and/or the leaching of DOC from the soil.

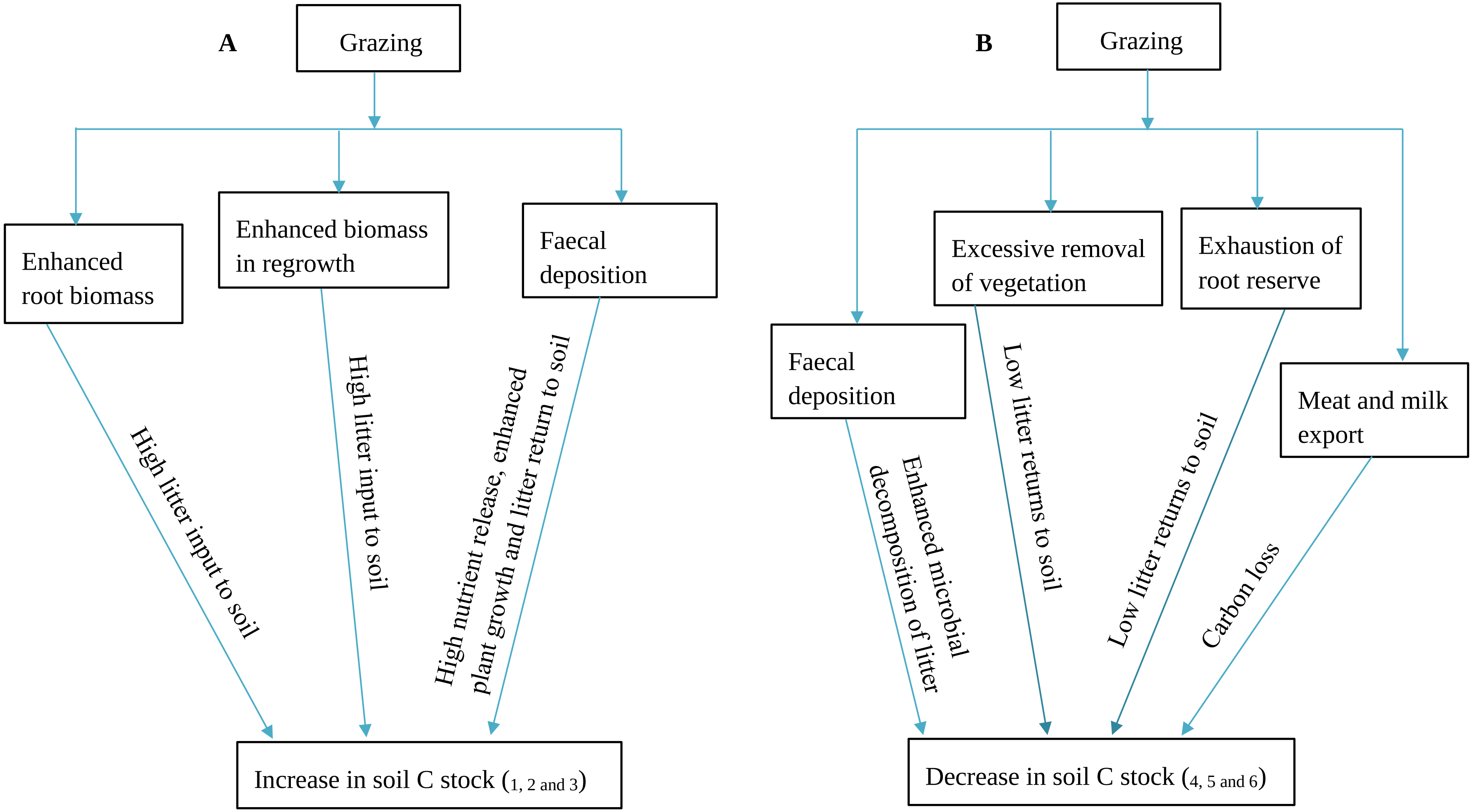


Figure 1.4: Mechanisms through which grazing enhances (A) or depletes (B) soil carbon (C) stock. 1 = Conant and Paustian, 2002; 2 = Frank *et al.*, 2002; 3 = Chen *et al.*, 2015; 4 = Meyles *et al.*, 2006; 5 = Mestdagh *et al.*, 2006; 6 = Clay and Worrall, 2013.

1.2.4 Net management effects on soil carbon storage

The preceding sections showed that the response of soil C to grazing regimes and grazing-related management activities such as fertilizer application and liming is a function of complex interactions of management and non-management factors such as climate, soil type and soil properties. This probably explains the contradictory effects of grazing (e.g. Dlamini *et al.*, 2016; McSherry and Ritchie, 2013; Piñeiro *et al.*, 2010; Zhou *et al.*, 2017), fertilizer application (Geisseler *et al.*, 2016; Liu and Greaver, 2010; Lu *et al.*, 2011; Yue *et al.*, 2016) and liming (Paradelo *et al.*, 2015) on SOC stock such that no change, an increase and a decrease in SOC stock have been reported in grassland studies around the world. Also, in reality, management of grasslands can range from extensive practices such as no/minimal chemical inputs and occasional grazing to intensive practices such as regular chemical additions including lime, N, P and K containing fertilizers, and year-round grazing (Tiemeyer *et al.*, 2016). The combination of different management activities in one site could have combined effects that are different from the effects of each of the separate management activities. Thus, there is need to study both the separate and the combined effects of management activities on SOC under specific climate and soil conditions. This will help in devising management practices that are site-specific and are effective in achieving targeted ecosystem services.

1.3 Climate change and soil carbon storage in grasslands

The global climate is changing and the evidence is now clearer and more reliable. For example, IPCC (2014) reported that the warming of the global climate system is unequivocal and the global average temperature had risen by nearly 0.8°C since the late 19th Century (i.e. 1880), with about 0.2°C per decade increase since the 1980s. Relative to 1986 – 2005 baseline, global mean surface temperature has been projected to increase by 0.3 – 0.7°C between 2016 and 2035, and 1.7 – 4.8°C by the end of the 21st Century (2081-2100) (IPCC, 2014).

Climate influences the size of the grassland soil C store through its control on plant growth, and therefore rates of litter and plant exudate inputs to soil and the rates of C loss through decomposition, leaching and erosion (Albaladejo *et al.*, 2013; Bellamy *et al*., 2005; Rees *et al*., 2005). Due to this coupling of the C cycle to the climate system, climate change could therefore lead to a net soil C gain or loss depending on the balance between the processes discussed in the following sections.

1.3.1 Soil carbon gain under climate change

In the temperate regions, temperature increase at either end of the summer can result in soil C gain by extending the length of growing season, thereby enhancing plant growth and C additions to the soil (Chang *et al.*, 2016). For example, Xia *et al.* (2014) observed that increasing spring temperature at latitudes between 30 and 90 °N has largely enhanced ecosystem productivity. They explained that spring warming stimulates the onset of leaf unfolding which results in an earlier start of the growing season. A caveat to this pathway of climate change-induced soil C gain is that changes in the phenology of plant communities as a result of changing growing season could disrupt the existing ecological interactions (Bardgett *et al.*, 2013). This may affect the expected increase in soil C accumulation.

Soil C gain may also result from increased annual precipitation, especially if the distribution of precipitation is sufficient during the growing season. The increase in both annual and growing season precipitation has been associated with enhanced plant productivity (Ni, 2004; Nippert *et al.,* 2006), which supplies C to the soil. In a six-year experimental climate change study, He *et al.* (2012) found that 30% increase (+120 mm) in annual precipitation resulted in a significant increase in SOC stock (+145.9 g C m^{-2} within 30 cm depth) of Inner Mongolian grasslands. Also, the weathering of primary minerals and the formation of clay colloids (which increase with precipitation, although over a long period of time) increase the soil surface area available for the adsorption of C substrates; these adsorbed materials are protected from microbial decomposition (Davidson, 2015). The higher the amount of C in the soil that is not available to microbial decomposition, the higher the tendency for a net C gain in the soil. Many studies (e.g. Follet *et al.,* 2012; Loveland and Webb, 2003; Sausen *et al.,* 2014) have shown that soils with higher clay content are generally higher in SOC, if management and land use are similar.

Increasing CO_2 concentration in the atmosphere has a fertilization effect on plants which increases their growth as well as C addition to the soil (Norby *et al.,* 2005; Zak *et al.,* 2011). This is possible because the increased atmospheric CO_2 enables plants to take up more molecules of CO_2 per molecule of water transpired thereby increasing their net primary productivity (Bala *et al.,* 2013). Xu *et al.* (2014) assessed the effects of elevated CO_2 on plant growth in Chinese grasslands. They found that elevated CO_2 resulted in a significant increase (11 – 52%) in plant growth. However, a meta-analysis of 77 grassland studies shows that soil N availability is essential for the elevated CO_2-induced plant growth to enhance soil C storage (Sillen and Dieleman, 2012). It has also been argued that the radiative effects of increasing atmospheric CO_2 dampen the fertilization-induced C uptake in the terrestrial ecosystem (Bala *et al.*, 2013). Also, the increased labile C allocation to the soil due to increased atmospheric CO_2 concentration (Phillips *et al.*, 2009) may have a priming effect on dormant soil microbes thereby stimulating the decomposition of 'old' C stock (Heimann and Reichstein, 2008). This further reduces any net soil C gain potential of increasing atmospheric CO_2. Similarly, Higgins and Harte (2012) have challenged the idea of a linear relationship between atmospheric CO_2 levels and C storage in plants and soils. They argued that only short term leaf and plot-level experiments in a few ecosystems support this view. They also argued that the influence of factors such as changes in temperature and precipitation were not accounted for in these experiments. Therefore, the potential for increasing atmospheric CO_2 concentration to enhance soil C storage is highly uncertain (Higgins and Harte, 2012).

1.3.2 Soil carbon loss under climate change

The major process of soil C loss is through microbial decomposition of OM or soil microbial respiration, which releases about 60 Pg C year^{-1} globally (Shao *et al.*, 2013). This efflux of C from the soil due to microbial respiration is significantly stimulated by warming (Peng *et al.*, 2015). As temperature increases, the rate of soil microbial decomposition of OM increases; and this results in the loss of soil C as CO_2 or CH_4 into the atmosphere (Ward *et al.*, 2013). However, how long the effects of increasing temperature on OM decomposition will last is still uncertain. There is no consensus on the exact effect of increasing temperature on the temperature sensitivity (or more specifically temperature quotient-Q_{10}) of SOM decomposition. The Q_{10} is the factor of increase in SOM decomposition with a 10° rise in temperature (Von Lützow and Kögel-Knabner, 2009). Under rising temperature, the Q_{10} may increase, decrease or remain unchanged depending on the effects of some constraining environmental factors such as the availability of readily decomposable substrates (Kirschbaum, 2004) and microbial access to substrates (Moinet *et al.*, 2018). This suggests that increasing temperature may not always lead to an increase in soil C loss. Crowther and Bradford (2013) are of the view that thermal acclimation, which is the physiological adjustment by soil microbes to compensate for initial temperature change, may occur thereby making any temperature-induced increase in soil C efflux only transitory. Other changes in soil microbial community induced by increasing temperature, such as stimulating genes for degrading labile C without effect on recalcitrant C, have also been suggested to maintain soil C stock under warming conditions (Zhou *et al.,* 2007; Zhou *et al.,* 2012).

Soil characteristics also influence the response of soil C to temperature change. For example, microbial access to substrates, which influences the Q_{10}, is significantly affected by soil structure and texture. Substrates become unavailable to microbes in well-aggregated soils or soils with high clay content because the organic materials get occluded within the aggregates or adsorbed on the surfaces of clay fractions. This reduces the rate of SOM decomposition even under increasing temperature (Davidson and Janssens, 2006). In addition to the adsorption of OM by clay particles, soils with high amounts of clay influence the effects of temperature on OM decomposition by regulating soil moisture content. Soil moisture content increases with rainfall and the amount of clay due to: 1) large proportion of micro pores in clay-dominated soils that are discontinuous, and 2) the high electrically charged surfaces of the clay particles bind soil particles and water molecules, thereby limiting drainage (Jarvis, 2007). In wet soils, an increase in evapotranspiration due to increasing temperature tends to favour OM decomposition (Bellamy *et al.,* 2005). This happens because evapotranspiration alters the soil water potential and redox status in favour of aerobic respiration (Hartmann and Niklaus, 2012). In soils with moisture limitation, the input of plant photosynthates to the soil and the activities of exo-enzymes that are necessary for OM decomposition are significantly reduced (Gorissen *et al.,* 2004; Sanaullah *et al.,* 2011), hence, increasing temperature under dry soil condition may not have significant effects on OM decomposition. The effect of temperature increase on decomposition is also moderated by nutrient availability (Davidson *et al.,* 2000; Jentsch *et al.,* 2011). For example, under limited N availability, fungi use lignin as N source; this increases the rate of decomposition of recalcitrant substrates and results in C loss (Heimann and Reichstein, 2008).

In addition to soil microbial respiration or OM decomposition, soil C is also lost through leaching and erosion (Rees *et al.,* 2005). Generally, the processes of leaching and erosion increase with an increase in precipitation thereby enhancing the loss of soil C into surface waters, mostly as dissolved and particulate C (Dawson and Smith, 2007). This pathway of soil C loss is complicated as it interacts with other factors. For instance, both leaching and erosion are influenced by soil characteristics. In a predominantly clay soil, with limited drainage, the rate of leaching is often impeded. Similarly, on a flat terrain with poorly drained soils; the soils get waterlogged with increasing precipitation, and erosion tends to be minimal. Under this waterlogged soil condition with limited leaching and erosion, soil C tends to accumulate. The caveat to this is that C could be lost in this condition through anaerobic release of CH_4, which is the second most important greenhouse gas after CO_2 contributing to global warming. Globally, anaerobic soil respiration in wet soils release 0.04 – 0.16 Pg CH_4 year^{-1} into the atmosphere (Petrescu *et al.,* 2010).

1.3.3 Net effect of climate change on soil carbon storage

Climate manipulation experiments have been set up to investigate the effects of climate change on grassland C dynamics (e.g. De Boeck *et al.,* 2007; Grime *et al.,* 2000; Selsted *et al.,* 2012). A synthesis of data from these experimental studies (see Ni *et al.,* 2017) shows that warming increased both soil C input and loss with no significant effect on net C pool, and whereas increased precipitation stimulated soil C input, drought suppressed it. The interactive effects of changing temperature and precipitation on C cycling in grasslands remains poorly understood (Lei *et al.,* 2016). This requires urgent research attention as the interactive effects of warming and altered precipitation may differ from their simple additive effects (Ni *et al.,* 2017; Wu *et al.,* 2011). There are also combined effects of climate change and management activities on grassland ecosystems (Carlyle *et al.,* 2014) which need to be considered in climate change studies. Thébault *et al.* (2014) used a meta-analysis of 38 published manipulative experiments to show that the interactions between management practices and climate change provided better explanation for observed ecosystem changes than any of the factors in isolation.

The need to investigate climate change effects on managed grasslands is a particular concern for UK uplands (see Section 1.4), which have witnessed changes in climate that are much greater than in the lowlands (House *et al.,* 2010). For example, between 1961 and 2000, minimum temperatures increased more than maximum temperatures in the uplands, whereas there was no difference in the changes between minimum and maximum temperatures in the lowlands (Burt and Holden, 2010). Morecroft *et al.* (2009) also found that temperature trends between 1993 and 2007 differed between upland and lowland sites in the UK, with an average temperature increase of 1.2°C in the uplands and 0.7°C in the lowlands. A more detailed description of the UK uplands is given in the next section.

1.4 The UK uplands

In general, the UK uplands are areas that are over 250 m above sea level, include all land above the upper reaches of enclosed farmlands and cover a third of the land surface of the UK (JNCC, 2009; Reed *et al.,* 2009). The climate of the uplands is generally cold and wet, with an east to west gradient of about 1000 mm to 3000 mm of annual rainfall and a short growing season of between 150 and 200 days (Reynolds and Edwards, 1995). Mean winter temperatures in many of the uplands are close to zero while mean annual temperatures (MAT) range from around 1.6 to 8.0°C (Burt and Holden, 2010). There is a decrease of about 0.65°C in temperature with every 100 m increase in elevation (Averis *et al.,* 2004). The uplands are a vital source of high quality waters with low resultant water treatment costs (House *et al.,* 2010; Stevens *et al.,* 2008). However, there is currently growing evidence of an increase in the DOC content of upland waters (Evans *et al.,* 2006; Yallop *et al.,* 2010), which perhaps indicates losses from within the soil C stores (Buckingham *et al.,* 2014; Freeman *et al.*, 2001). Although DOC is not directly harmful, it is associated with water colour and can react with chlorine during water treatment to produce trihalomethanes, which are potential carcinogens; also, the removal of DOC in water is costly (Stevens *et al.,* 2008).

1.4.1 Upland soils

Organic (peat) and organo-mineral (stagnopodsols, stagnohumic gleys and acid brown earths) soils dominate the uplands (Table 1.3). This predominance of organic-rich soils can be attributed to the interaction of topography, climate and parent rocks (Holden *et al.,* 2006). The uplands are cold and wet, and together with low rates of evapotranspiration contribute to highly leached and poorly weathered acidic soils (Floate, 1977). The strong acidity and low temperatures contribute to slow decomposition of organic materials and the accumulation of OM (Bishop *et al.,* 2008; Floate, 1977). The flat plateaus are mostly covered with thick blanket peat, whereas on gentle slopes the organic surface layers are thinner and overlay mineral soils, thus giving organo-mineral soils such as stagnopodsols (Avery, 1980).

Table 1.3: The main organic-rich soil types of UK uplands and some of their characteristics (Newbould, 1985; Holden *et al.*, 2006).

Soil	Soil group	Defining characteristics	pH	Soil carbon (% of total in organic soils) in England and Wales	Main species of grass
Peat	Raw peat (Blanket peat)	Undrained organic soil (> 50% OM) that is > 40 cm thick within 80 cm profile, and always wet to within 20 cm of the surface.	<4.0	51.2	*Calluna vulgaris, Erica tetralix, Trichophorum caespitosum, Molinia caerulea.*
	Earthy peat (Fen peat)	Relatively well-drained and aerated organic soil (> 50% OM) that is > 40 cm thick within 80 cm profile.	3.5-4.5	9.6	
Organo-mineral	Stagnopodzol	Soil with peaty surface layer < 40 cm thick, bleached and iron-rich subsurface horizon.	3.8-4.5	8.0	*C. vulgaris, Vaccinium myrtillus, E. cinerea, E. tetralix, T. caespitosum.*
	Stagnohumic gley	Soil with peaty surface layer at least 15 cm thick, greyish and mottled subsurface mineral horizon.	4.0-5.2	21.2	*M. caerulea, Festuca ovina, Deschmpsia flexuosa, V. myrtillus, Nardus stricta.*
	Brown earth	Non-alluvial loamy soil with dark-coloured surface horizon of variable depth that is rich in mull humus and mixed with brown-coloured subsurface mineral horizon.	4.6-5.5		*Agrostis tenuis, Trifolium repens, F. rubra, A. cavina, F. ovina,*

1.4.2 Farming in the uplands

Farming in the UK uplands is economically marginal because of limitations such as high rainfall, low temperature, short growing season, high elevation, steep slope, rockiness, poor drainage, soil acidity and nutrient deficiency (Floate, 1977). Farming in these uplands therefore relies heavily on agricultural subsidies (Dougill *et al.,* 2006). The dominant agricultural activity is livestock production which depends on semi-natural grassland and heathland habitat (Medina-Roldán *et al.*, 2012; Stevens *et al.*, 2008), with sheep being the dominant livestock (Holden *et al.*, 2006; Stevens *et al.*, 2008). Grazing activities usually also include cutting and storage of forage as hay or silage (Smith *et al.*, 2014; Soussana *et al.*, 2007). The major strategies for improving productivity in these upland grasslands include drainage (Avery *et al.,* 1995), re-seeding with ryegrass and clover species (Bullock *et al.,* 2011), and the addition of lime (Rangel-Castro *et al.,* 2005) and fertilizers (Floate, 1977). As a result of these improvement strategies, large areas of semi-natural vegetation in the uplands have been converted to improved pasture since the early 19th Century (Eadie, 1985; Hornung *et al.,* 1986).

Management of the uplands has changed overtime due to economic reasons and government policies (Holden *et al.*, 2007). Between 1945 and 1980, the government encouraged agricultural intensification in the uplands by giving financial incentives to farmers to increase the productivity and fertility of soils in order to support livestock production (Reed *et al.,* 2009). These incentives included subsidies to cut drainage ditches, particularly following the Second World War (Holden *et al.*, 2004), and stable market prices for agricultural products via the European Common Agricultural Policy (CAP) in the 1960s (Chang *et al.,* 2016). The resultant effects of agricultural intensification in the uplands include losses in hay meadow communities (Jefferson, 2005), loss of species-rich grasslands (Kirkham *et al.*, 2014), soil erosion and loss of some habitats (Harrod *et al.*, 2000; Ross *et al.*, 2003), changes in vegetation cover (Holden *et al.,* 2006), changes in grassland composition (Hopkins *et al.,* 1988), soil and habitat degradation (Reed *et al.,* 2009).

In order to reverse these negative effects of agricultural intensification, many measures have been taken. These include the introduction of agri-environment schemes which encourage farmers to revert to less intensive management practices (Coates, 1997; Taylor and Morecroft, 2009) and reforms in the European CAP (Reed *et al.,* 2009). The changes in EU policies that affected livestock production include the Nitrate Directive of 1991 that restricted nitrate fertilizer application in vulnerable zones thereby capping stocking density in pastures, and the shift in the incentives of CAP in 1992 by decoupling payments from production (Chang *et al.*, 2016). These changes in policy due to environmental concerns and changing market conditions caused a rapid decline in the number of sheep in UK uplands (SAC, 2008). However, some of the improved pastures are still sustained via fertilizer application and liming (Holden *et al.*, 2006). There is limited understanding of how the different management practices in the upland grasslands affect soil C dynamics, and how this may change with climate change. This thesis was therefore designed to fill that research gap and provide information that will help in formulating future grassland management policies.

This thesis is also timely as it will provide strong evidence for new agricultural land management policies in the UK following the UK's decision to leave the European Union (hereafter referred to as BREXIT). The UK is already in the process of leaving the EU and its CAP, and the future of UK agricultural policy is uncertain. The Department for Environment, Food and Rural Affairs (DEFRA) UK opened a consultation (27th February 2018 to 8th May 2018) on their proposed new agricultural policy which aims to reward land management activities that enhance the provision of public goods, which include flood mitigation, water and soil quality, atmospheric C sequestration to mitigate climate change, and enhanced biodiversity (DEFRA, 2018). This means that farmers would continue to manage the land for food production, but also be paid for the provision of a variety of public goods where they can be shown to be achievable. The evidence-base on how land management activities have worked to protect soil C stock in upland grasslands or enhance atmospheric C sequestration is not well defined. This means that the business case for land owners to deliver public goods is not clear, which in turn will hinder access to new funding schemes and capacity building efforts to support the delivery of public goods. This thesis will help fill the evidence gap by providing information on how different existing grassland management activities affect soil C sequestration in different climatic zones globally, and specifically within the UK context. The thesis will also explore how climate change will influence the effects of the grassland management activities on soil C dynamics. This will help inform the adoption of management activities that will be sustainable even in the event of climate change.

1.5 Research questions and objectives

As discussed previously in this chapter, climate change (Bellamy *et al.,* 2005) and land management (Holden *et al.,* 2007) are two critical factors affecting C dynamics in grasslands. The separate impacts of these two factors on C dynamics is still debated, more so, their interactive effect is much less understood (Carlyle *et al.,* 2014). Grassland C dynamics involve many processes such as fixing of atmospheric C in plants, translocation of C from plant biomass to the soil, release of C to the atmosphere via soil and plant respiration, storage of C in soil, distribution of soil C into different fractional pools, leaching and erosion of soil C, and C offtake by grazing animals and hay/silage cutting. All these processes are influenced by both climate change and soil management. It is nearly impossible to investigate all these complex interactions in one study, hence this thesis focused mainly on the following components of the C cycle: plant biomass productivity, net ecosystem C uptake, and soil C storage. The key questions that guided the thesis were:

1) What are the effects of inorganic fertilizers, liming and grazing on SOC stock in different climatic settings?

2) What are the effects of upland grassland management regimes on SOC stock and its fractional distribution?

3) What are the effects of upland grassland management regimes on net ecosystem C flux?

4) What are the effects of experimental warming and altered precipitation on plant biomass production and net C fluxes in upland grasslands?

The overall aim of this thesis was to investigate the impacts of climate change and management regimes on the C dynamics of UK upland grassland soils. The specific objectives were:

1) To determine the effects of inorganic fertilizer application, liming and grazing on grassland SOC stock in different climatic settings, based on published studies from across the globe,

2) To determine the effects of conventional pasture and traditional hay meadow management regimes on SOC stock and fractions in selected UK upland grasslands,

3) To assess the response of ecosystem CO_2 and CH_4 fluxes to conventional pasture and traditional hay meadow management regimes in selected UK upland grasslands, and

4) To assess the effects of experimental warming, supplemental precipitation and drought on plant biomass production, MBC, NEE, and DOC flux in selected UK upland grasslands.

It is expected that this work would help inform appropriate management policies for sustainable provision of multiple ecosystem services, particularly C sequestration and enhanced plant biomass production for feeding livestock. It is also expected that the information made available in this thesis will provide an evidence base to support the development of a new agricultural policy in the UK, post-BREXIT.

1.6 Research approach

Different research approaches were employed in this thesis. Firstly, a systematic literature review and data modelling were used to synthesize the effects of management activities on SOC stock in global grasslands (Objective 1, Chapter 2), to help place results from the UK in a global context. An observational study was carried out to assess the stock of organic C in both bulk soils and soil fractions in selected UK upland grasslands under different management regimes (Objective 2, Chapter 3). In the same UK upland grasslands, CO_2 and CH_4 fluxes were monitored over a year (Objective 3) and reported in Chapter 4. An experimental study was carried out to assess the impacts of climate change on plant biomass productivity and net C fluxes in soil cores with intact vegetation extracted from selected UK upland grasslands (Objective 4, Chapter 5).

1.6.1 Study area

Grasslands within the Yorkshire Dales, an upland area of the Pennines in Northern England, UK, were studied to achieve Objectives 2 - 4. Two locations, Nidderdale (54°09'N, 01°53'W) and Ribblesdale (54°05'N, 02°16'W) (Figure 1.5), at an altitude of approximately 300 m above sea level and a distance of about 20 km apart, were studied. These two locations were chosen because of their contrasting soil parent material (siliceous stones and limestone, respectively) and therefore soil type, which I expected to affect soil properties differently. The Nidderdale site is characterized by stagnohumic gley soils (Humic Gleysol) and formed from clay drift with siliceous stone content, whereas the Ribblesdale site is underlain by brown earth soils (Eutric Cambisol) formed from carboniferous limestone. At each location, there are two contrasting grassland management regimes: traditional hay meadow managed under an agri-environment scheme (both locations) and conventional pasture cut for silage (Nidderdale) or permanent grass continuously grazed by sheep (Ribblesdale). The traditional hay meadows under agri-environment schemes are less intensively managed with the aim of restoring, protecting and enhancing biodiversity, whereas the conventional pastures are managed primarily for livestock production.

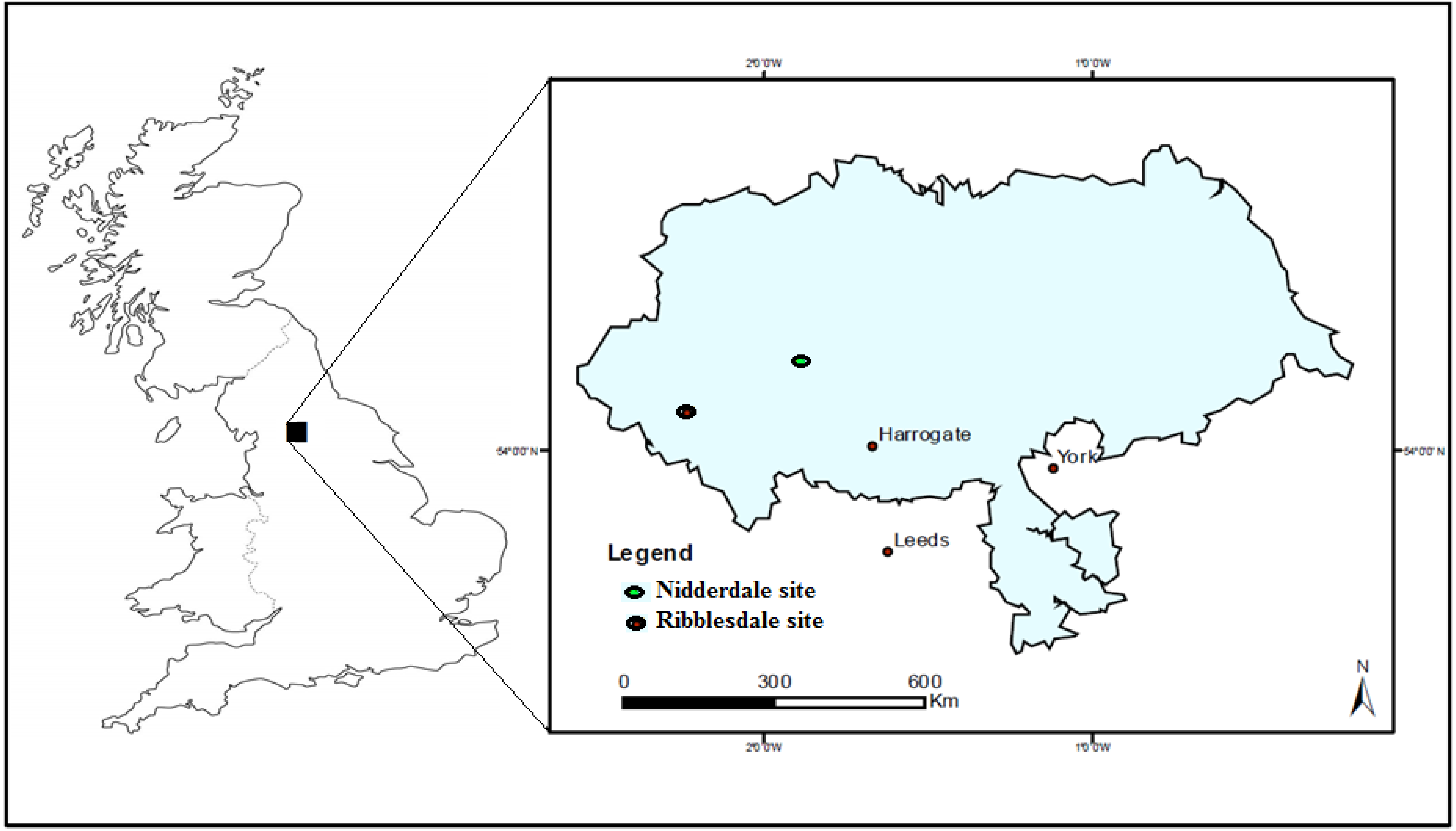


Figure 1.5: Map of Northern Yorkshire in the UK showing the study sites (Nidderdale and Ribblesdale).

The UK sites have a cool and wet climate with MAT of 7.4°C and mean annual rainfall of 1550 mm (1981 – 2010, recorded at Malham Tarn station located 6 km from Ribblesdale and 18 km from Nidderdale). The dominant grasses at both locations are *Holcus lanatus* and *Lolium perenne*. Other grasses and herbaceous species that are common at the Nidderdale sites are *Ranunculus repens* and *Trifolium repens*, whereas the Ribblesdale sites have *Anthoxanthum odoratum, Geranium sylvaticum, Poa trivialis, Festuca rubra, Deschampsia cespitosa* and *Alopecurus pratensis*.

1.6.2 Outline of research methodology

This section gives a brief outline of the rationale for the approaches used to achieve each research objective. The research techniques are described in Chapters 2 to 5. To achieve Objective 1, a meta-analytical approach was used. Meta-analysis is an effective quantitative approach used to summarise the effects observed across different studies (Hedges *et al.*, 1999). This approach was chosen because there was insufficient resources available to conduct an extensive field study, and the approach helped to synthesize the effects of liming, fertilizer addition and grazing regime on SOC stock globally and in different climatic zones. The meta-analytical approach estimates effect sizes as single values regardless of the number of datasets and studies used, and this makes the interpretation of net effects easier. This advantage makes the meta-analytical approach preferable to qualitative literature review, where the number of statistically negative and positive results are counted and weighed against each other, with no reliable means of determining net effects (Koricheva *et al.*, 2013). Although understanding the impacts of climate change and land management on C dynamics of UK upland grasslands was the main aim of this thesis, putting it within the global perspective was considered helpful to understanding the relative contributions of climate, environment and management as drivers of SOC stock. This was the reason for extending the meta-analysis beyond UK upland grasslands.

Objective 2 was achieved using a SOC fractionation technique that combines physical and chemical methods as proposed by Zimmermann *et al.* (2007). In general, physical methods involve separation on the basis of soil particle or aggregate size, density and magnetic properties, and chemical methods are based on hydrolysability, oxidizability and solubility of the SOC fractions (Priyanka and Anshumali, 2016; Von Lützow *et al.*, 2007). The method I used in this thesis combines aggregate size and density (physical methods) and oxidation by sodium hypochlorate (chemical method) to identify five distinct SOC fractions that were subsequently grouped into chemically protected, physically protected and labile C. The method I used has been recognized as "best practice" (Stockmann *et al.,* 2013). This is because it accounts for the influence of soil particles and aggregates as well as the chemical composition of organic C on the stability of SOC stock. The method used in this thesis is also increasingly being used by others (Dondini *et al.,* 2009; Poeplau *et al.,* 2013; Xu *et al.,* 2011) because the SOC pools it generates are comparable with conceptual SOC pools used in modeling soil C dynamics (Xu *et al.,* 2011).

A static chamber technique of greenhouse gas (GHG) measurement was used to address Objective 3. CO_2 and CH_4 fluxes were measured in the selected UK upland grasslands in order to assess the current C sink-source status of the grasslands and how they are affected by different management regimes. Although there are other micrometeorological or tower-based techniques (e.g. eddy covariance and Bowen ratio) which have the advantage of continuous measurement of GHG fluxes in the field, the chamber technique was used in this thesis for two major reasons. Firstly, the chamber technique is less expensive than the micrometeorological techniques and was therefore affordable. Secondly, the chamber technique measures localised (centimetre to meter scale) GHG fluxes, hence, it accounts for spatial variability in both soil and vegetation (Balogh *et al.,* 2007). In contrast, the eddy covariance measures CO_2 flux at a larger scale of hectares to several square kilometres (Baldocchi, 2003), which makes it unsuitable for accounting for small scale variability in fluxes within the sites that I studied. Also, the eddy covariance technique is more suitable for measuring CO_2 fluxes when the atmosphere is stable and over a horizontally homogenous flat terrain (Baldocchi, 2003), which renders it less suitable for use on exposed, upland hillslopes.

Objective 4 was achieved through a climate manipulation experiment. Soil cores with intact vegetation were extracted from selected UK upland grasslands and subjected to three months of experimental warming, drought and supplemental precipitation, based on the projected summer climate for the site by the end of the 21st Century. Climate manipulation experiments are used to study the impacts of changing climatic variables on ecosystem processes. The experiment can be done in field conditions where rain-out shelters are used to control rainfall and heaters are used to manipulate temperature (e.g. Poll *et al.,* 2013). Although the field experiments may give a realistic view of ecosystem responses to climate change, underlying mechanisms often are not well understood and desired changes in climatic variables are often not achieved (Krab *et al.*, 2015). To better understand the underlying mechanisms of ecosystem response to specific changes in climate, homogenized soil and/or litter are incubated in microcosms under controlled laboratory conditions (e.g. Chang *et al.,* 2012). However, the laboratory experiments with homogenised soils oversimplifies the complex processes in field conditions and may present unrealistic results (Krab *et al.,* 2015). In this thesis, field manipulation experiments could not be carried out due to limited resources. Also, incubation of homogenised soil and litter samples was not done due to the concern of oversimplification of ecosystem processes. The approach used in the thesis i.e. subjecting soil cores with intact vegetation to controlled climate conditions in the laboratory, offered a compromise and allowed the complex soil-plant interactions in the field to be preserved and the target climate conditions to be achieved.

1.7 Thesis outline

This thesis consists of six chapters, with Chapters 2 – 5 providing answers to the four research questions that guided the thesis (Figure 1.6). Chapter 1 provides an overview of available literature, which gave context to the thesis and established its relevance. In Chapter 1, research gaps were identified and the rationale for the study explained. Research questions, objectives of the study and research methodology are also outlined.

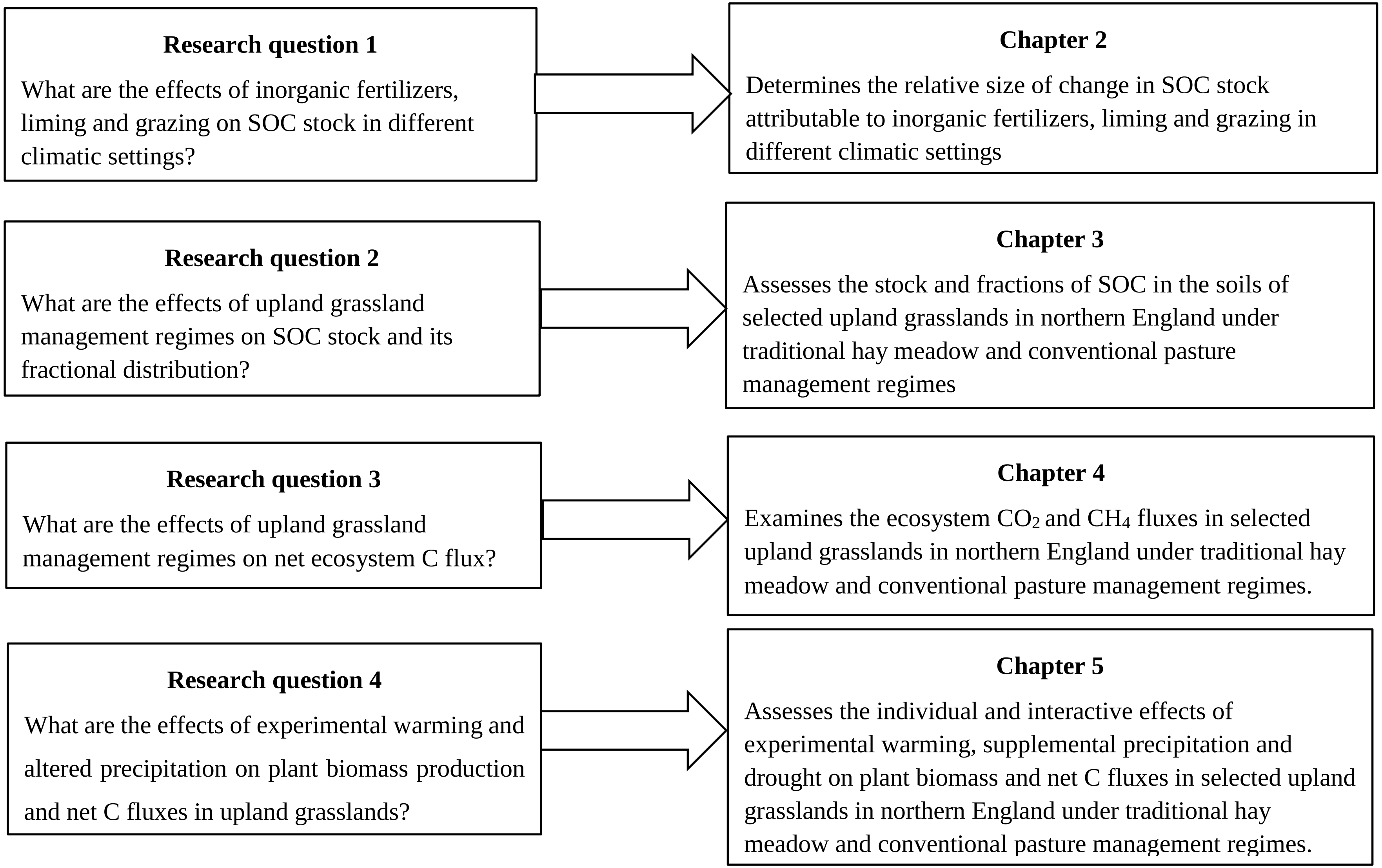
Chapter 2 investigates the effects of fertilizer application, liming and grazing on SOC stock in different climate settings. Chapter 2 also provides an overview of the response of temperate grassland SOC stock to management activities under different temperature and precipitation regimes.

Chapter 3 assesses the effects of two important management regimes of UK upland grasslands (traditional hay meadow under agri-environment schemes and conventional pasture) on the stock and fractional distribution of SOC.

Chapter 4 examines the effects of the same two management regimes (traditional hay meadow under agri-environment schemes and conventional pasture) on the CO_2 and CH_4 fluxes.

Chapter 5 investigates the potential effects of projected summer climate on the plant biomass production, net C uptake, MBC and DOC fluxes of one of the UK upland grassland locations studied in previous chapters.

Chapter 6 is a synthesis of the main findings in this thesis. It draws together the findings in Chapters 2 – 5, and discusses the wider implications of the findings. The limitations of the study and directions for future work are also discussed. The chapter ends with a summary of the conclusions from the thesis.



Research question 1

What are the effects of inorganic fertilizers, liming and grazing on SOC stock in different climatic settings?

Chapter 2

Determines the relative size of change in SOC stock attributable to inorganic fertilizers, liming and grazing in different climatic settings

Research question 2

What are the effects of upland grassland management regimes on SOC stock and its fractional distribution?

Chapter 3

Assesses the stock and fractions of SOC in the soils of selected upland grasslands in northern England under traditional hay meadow and conventional pasture management regimes

Research question 3

What are the effects of upland grassland management regimes on net ecosystem C flux?

Chapter 4

Examines the ecosystem CO_2 and CH_4 fluxes in selected upland grasslands in northern England under traditional hay meadow and conventional pasture management regimes.

Research question 4

What are the effects of experimental warming and altered precipitation on plant biomass production and net C fluxes in upland grasslands?

Chapter 5

Assesses the individual and interactive effects of experimental warming, supplemental precipitation and drought on plant biomass and net C fluxes in selected upland grasslands in northern England under traditional hay meadow and conventional pasture management regimes.

Figure 1.6: Research questions and the chapters providing answers.

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Chapter 2: Soil organic carbon stock in grasslands: effects of inorganic fertilizers, liming and grazing in different climate settings

In review in Journal of Environmental Management

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Abstract

Grasslands store about 34% of the global terrestrial C and are vital for the provision of various ecosystem services such as forage and climate regulation. About 89% of this grassland C is stored in the soil and is affected by management activities but the effects of these management activities on C storage under different climate settings are not known. In this study, we synthesized the effects of fertilizer (nitrogen and phosphorus) application, liming and grazing regime on the stock of SOC in global grasslands, under different site specific climatic settings using a meta-analysis of 341 datasets. We found an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, mainly attributable to grazing (-15.0%), and only partially attenuated by fertilizer addition (+6.7%) and liming (+5.8%), indicating that management to improve biomass production does not contribute sufficient organic matter to replace that lost by direct removal by animals. Management activities had the greatest effect in the tropics (-22.4%) due primarily to heavy grazing, and the least effect in the temperate zone (-4.5%). The negative management effect reduced significantly with increasing mean annual temperature and mean annual precipitation in the temperate zone, suggesting that temperate grassland soils are potential C sinks in the face of climate change. For a sustainable management of grasslands that will provide adequate forage for livestock and mitigate climate change through C sequestration, we recommend that future tropical grassland management policies should focus on reducing the intensity of grazing. Also, to verify our findings for temperate grasslands and to better inform land management policy, future research should focus on the impacts of the projected climate change on net greenhouse gas exchange and potential climate feedbacks.

Keywords: Soil carbon, grasslands, land management, climate change, carbon sequestration, liming, fertilizer, grazing.

2.1 Introduction

Grasslands cover approximately 40% of the earth's surface (excluding Antarctica and Greenland), are distributed across all continents and over a wide range of geological and climatic conditions (Suttie *et al.,* 2005; White *et al.,* 2000). About 34% of the global terrestrial C is stored in grasslands and a large (89%) amount of the C sequestered by the grassland vegetation is stored in the soil (Ajtay *et al.,* 1979; White *et al.,* 2000), which is vital for the provision of ecosystem services and particularly for climate regulation (Buckingham *et al.,* 2013).

The distribution and productivity of grasslands is mainly limited by climate and inherent soil properties. Globally, 28% of grasslands are distributed in semi-arid areas, 19% in arid areas, 23% in humid areas and 20% in cold areas (White *et al.,* 2000). Climate exerts an overriding influence on the size of the grassland soil C store through its control on plant growth, and therefore rates of litter and plant exudate inputs to soil and the rates of C loss through decomposition, leaching and erosion, and these processes are particularly sensitive to precipitation and temperature patterns (Albaladejo *et al.,* 2013; Bellamy *et al*., 2005; Rees *et al*., 2005). Currently, climate is changing, with nearly 0.8°C rise in global average temperature since the 19th Century and a greater warming as well as altered precipitation patterns expected throughout the 21st Century (IPCC, 2013; Jenkins *et al.,* 2008). Thus grasslands that naturally exist at the margins of their climatic and edaphic envelope, or whose continued existence depends on management activities may be particularly sensitive to climate change, with poorly understood consequences for soil C stocks and feedback to climate change.

Globally, grasslands are managed to increase biomass productivity in order to support livestock production, and are either being directly grazed, or cut for fodder, typically as hay or silage, or a combination of all three. Management activities are primarily used to change the status of soil properties thereby creating optimum conditions for plant growth.

Soil characteristics that have been associated with rapid grassland establishment and increased productivity include relatively high sand and silt and low clay contents, and therefore moderate drainage, friable consistency, small aggregates, slightly acidic condition, and high nutrient levels (Epstein, 2012; Fay *et al.,* 2012; Gibbs, 1980). Nutrient levels and acid status can be improved by fertilisation and liming to raise the soil pH, and these are typically the most common management activities for improving or maintaining grassland productivity. As well as the intended increase in aboveground biomass, fertilisation and liming potentially lead to greater production of root exudates and litter, and often have unintended effects on soil properties such as microbial populations and their activities that influence decomposition processes (Alonso *et al.,* 2012; Hoffman *et al.,* 2014; Soussana *et al.,* 2007). These management activities therefore have implications for soil C storage and sequestration.

Grazing regime itself may also influence net soil C storage. For example, soil C gain may result from over-compensatory plant growth (Tanentzap and Coomes, 2012) and increased inputs from enhanced root production (Frank *et al.,* 2002). Conversely, overgrazing could lead to soil C loss through reduced plant productivity and litter inputs (Conant and Paustian, 2002; Mestdagh *et al.,* 2006), or to exposure of bare soil and C loss via erosion (Evans, 1997). Thus a complex array of direct grazing effects and indirect grazing-related management effects on soil C storage may occur simultaneously. It is perhaps not surprising, therefore, that observed effects of liming, fertilizer application and grazing regime on soil C stock have been contradictory, and that increases, decreases and no change in soil C stock have been reported in different grassland ecosystems (Table A2.1) with specific climatic and soil conditions.

A number of global-scale reviews and meta-analyses have also reported inconsistent effects of grazing (Dlamini *et al.,* 2016; McShery and Ritchie, 2013; Pineiro *et al.,* 2010; Zhou *et al.,* 2016), fertilizer application (Geisseler *et al.,* 2016; Liu and Greaver, 2010; Lu *et al.,* 2011; Yue *et al.,* 2016), liming (Paradelo *et al.,* 2015) and grassland improvement (Conant *et al.,* 2001; 2017) on grassland soil C stock. For example, Zhou *et al.* (2016) reported a 10.28% grazing-induced reduction in soil C stock, whereas Pineiro *et al.* (2010) and McShery and Ritchie (2013) showed that grazing caused an increase, a decrease and no change in soil C stock with grazing effect size ranging from -0.33 to +0.38, depending on soil characteristics, climate and grazing intensity. Also, in separate analyses, N addition has been reported to cause a decrease (effect size = -0.0026; Lu *et al.,* 2011), no change (Liu *et al.,* 2010) and an increase (+19.75%; Yue *et al.,* 2016) in the C stock of grassland mineral soil layers. The differences in outcome could be attributed to a failure to account for context-specific differences in management, such as rates of fertilizer and lime application in different climatic zones (Dessureault-Rompré *et al.,* 2010; Iturri and Buschiazzo, 2016), or grazing regimes that vary depending on climatic influences on productivity (Oba *et al.,* 2000), or failure to consider the influence of soil type and characteristics (Mills *et al.*, 2005; Srinivasarao *et al.*, 2009).

The interactive effects of non-management factors (e.g. climate and soil) and fertilizer or lime application rates have not been synthesized for global grasslands. The few global studies (Dlamini *et al.,* 2016; McSherry and Ritchie, 2013; Zhou *et al.,* 2016) that considered interactive effects of grazing regime and non-management factors reported conflicting results. For example, McShery and Ritchie (2013) reported that grazing-induced changes in soil C stock were insensitive to either climate or soil texture, Dlamini *et al.* (2016) reported that significant soil C reduction due to over-grazing occurred only in cold (mean annual temperature, MAT < 0°C) and dry (mean annual precipitation, MAP < 600 mm) climates, and in acidic (pH<5.0) and coarse-textured (< 32% clay) soils, whereas Zhou *et al.* (2016) found a significant reduction in soil C only in semi-humid and humid regions (MAP ≥ 400 mm). In order to inform appropriate management decisions in global grasslands and models that integrate climate and land management, there is need to resolve the conflicting results of previous studies. This may be better achieved if the effects of site-specific characteristics and grazing-related management activities within different climatic zones are considered.

Our aim in this study is to investigate how grassland SOC stock responds to management activities in different climatic zones, and the influence of soil properties, in a single meta-analysis. Specifically, we determine the effect size (relative size of change in SOC stock) attributable to grazing-related management (liming and fertilizer addition) and grazing regime in different climatic settings, using a global meta-analysis approach. We focus on soil C stock rather than greenhouse gas inventory because understanding the fate of C stock is important not just for climate change mitigation but the provision of other ecosystem services such as maintaining soil quality, which is of immediate concern to farmers that manage the grasslands for livestock production. The result of this study will not only help to detect the overall pattern of response of SOC stock to major grassland management activities but also identify grasslands that are most likely to serve as either a C sink or a C source in the face of climate change. This will better inform policy decisions on future grassland management for sustainable provision of ecosystem services. We hypothesize that 1) the response of SOC stock to management activities will be significantly influenced by site-specific climatic setting and soil characteristics, and 2) fertilizer application, liming and grazing will result in an overall reduction in SOC stock.

2.2 Methodology

2.2.1 Data selection and extraction

All the data used for this study were extracted from peer-reviewed journal articles published before January 2017. A search for the articles was conducted in Web of Science between June and December 2016, using all combinations of the following groups of search terms: 1) management, liming, lime addition, fertilizer, nitrogen addition, nitrogen fertilizer application or grazing, 2) soil carbon, soil carbon stock, soil carbon storage or carbon sequestration, 3) grassland, pasture or meadow.

Our searches produced 2881 journal articles which we screened following a number of criteria: 1) they were grassland field studies in which SOC data (concentration in % or g/kg, stock in g/m^2 or Mg/ha, or both) were recorded in response to either liming, fertilizer application or grazing regime, 2) SOC data were recorded for both the managed field and a well-defined control field, and measurements were made at the same temporal and spatial scales, 3) only one of the target management practices such as grazing regime or nitrogen fertilizer varied while other management activities were absent or remained constant, 4) the depth of soil samples used for SOC determination were clearly specified, 5) the mean, sample sizes, measures of variability such as standard deviation, standard error or coefficient of variation can be extracted from the study, 6) experimental and control plots were established within the same ecosystem and had similar environmental characteristics at the beginning of the study, 7) management activities such as grazing intensity were clearly described quantitatively and/or qualitatively, and 8) experimental duration was clearly specified and was at least one entire growing season in order to avoid the effect of short term noise. In cases where two or more studies reported the same data from the same experiment, we chose one of the studies and excluded others, except if they provided supporting environmental information about the site. In order not to violate the key assumption of meta-analysis that studies must be independent, we chose data for the last year of sampling in studies where sampling was conducted annually from the same site. We excluded studies where either multiple nutrient fertilizers (e.g. NPK fertilizers) or organic manure (e.g. livestock slurry or industrial effluent) were applied. This was done to enable us to detect the exact effects of single nutrient fertilizers and prevent the confounding effects of high C and multiple nutrient contents of organic manures. We considered different management levels (e.g. different N levels or forms, or livestock stocking densities) sharing the same control plot as independent observations.

After a thorough screening, we selected 136 articles which yielded 341 pairs of independent studies (Tables S1 and S2), distributed among management activities as follows: 232 (grazing regime), 89 (fertilizer application) and 20 (liming). The selected studies, especially those on grazing, were distributed in most continents (Figure 2.1). Data was only available for N and phosphorus (P) fertilizers. We extracted data directly from tables or texts in the selected articles, or indirectly from figures using WebPlot Digitizer (http://arohatgi.info/WebPlotDigitizer/app/).

In addition to SOC, management, and duration data, we extracted data for the following characteristics when available: longitude, latitude, elevation, MAT, MAP, SOC at the beginning of the experiments (initial SOC), aboveground biomass (AGB), belowground biomass (BGB), clay content of the soil and soil bulk density. When MAT or MAP or either were not reported, we used ArcMap to extract the data from WorldClim-global climate database (http://worldclim.org/) with a spatial resolution of 30 arc seconds. Where only standard errors were reported, we converted them to standard deviations using the sample size.

Thirty–one studies did not report any measures of variability and we calculated their standard deviations following a method used by Geisseler *et al.* (2016). This involved calculating the average coefficient of variation (CV) across each management activity for all the datasets for which standard deviations were reported and using these average CVs to calculate the missing standard deviations. This was done separately for the control and the experimental datasets.

The equivalent soil mass method is recommended for comparing SOC stock changes in managed ecosystems (Lee *et al.*, 2009), in order to overcome the effect of compaction. This approach was not used here because not all the studies that we selected reported their

SOC data on such basis. However, we considered extent of sampling depth as a moderator in our meta-analysis and grouped our data into three depth categories: 0 – 19cm, 0 – 40cm and 0 – 100cm. In order to compare SOC stock across studies we converted reported SOC concentrations (%) to SOC stock (Mg/ha) using reported bulk density and sampling depth values as follows:

$$SOC\ stock\ (Mgha^{-1}) = SOC\ concentration\ (\%) \times Bulk\ density\ (gcm^{-3}) \times depth(cm) \qquad (2.1)$$

Thirty–one studies reported SOC only as concentrations in %, and did not report their corresponding bulk density values. To overcome this problem and maximise the number of studies available for meta-analysis, we estimated bulk density based on the relationship between SOC (%) and bulk density in all other studies (Figure A2.1). The best function with the highest coefficient of determination was exponential:

$$Bulk\ density\ (gcm^{-3}) = 1.3961e^{SOC\ concentration\ (\%)}\ (R^2 = 0.6246, p < 0.05) \qquad (2.2)$$

Bulk densities calculated with equation (2.2) were subsequently used to convert SOC concentrations to stock using equation (2.1). This approach has previously been used to calculate missing bulk density values (e.g. Hopkins *et al.*, 2009; Xiong *et al.*, 2016).

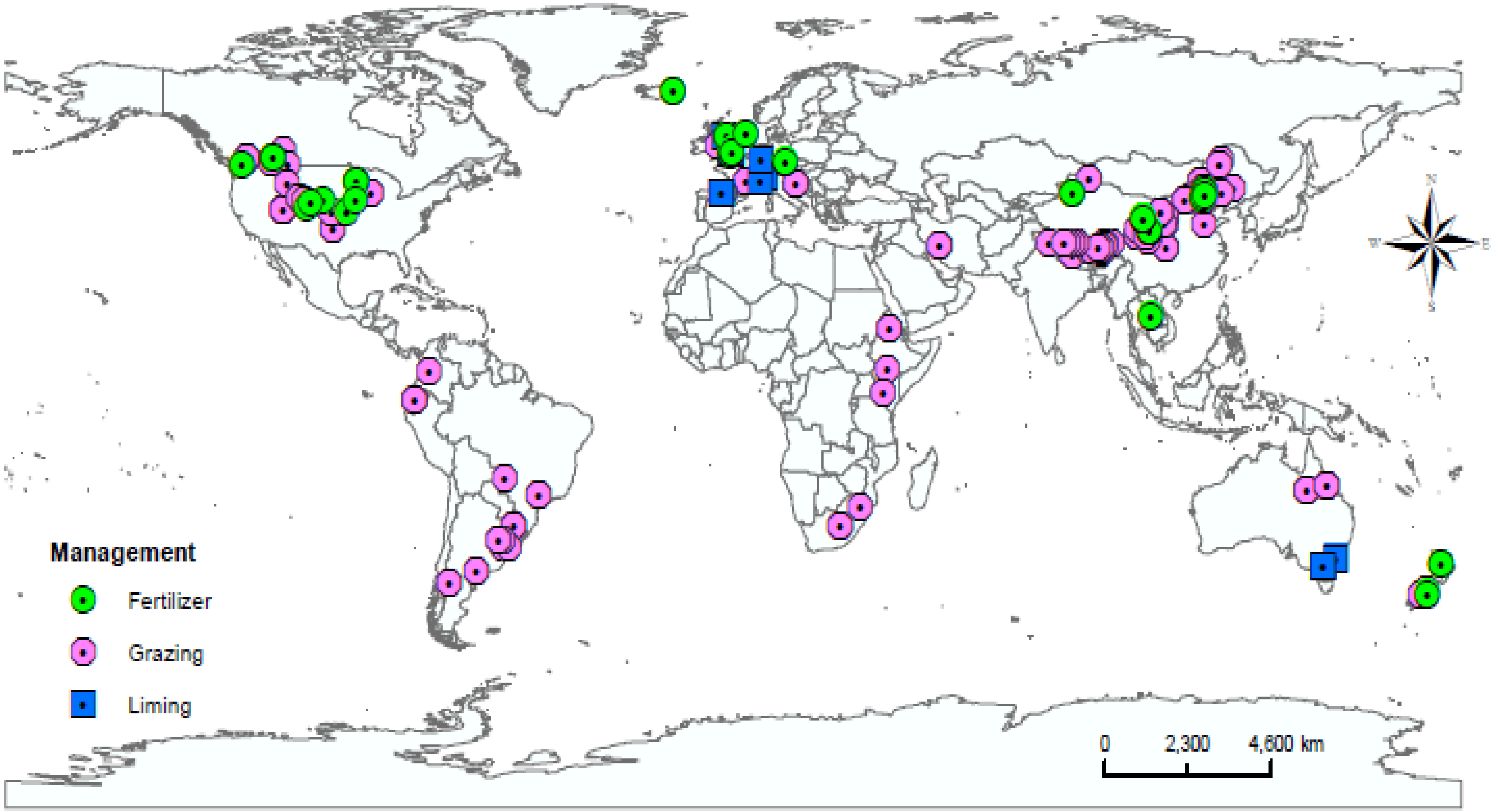

Figure 2.1: Global distribution of datasets used for meta-analysis.

The management activities (fertilizer application, liming and grazing) were divided into different categories so as to understand the variations within each management activity. We adopted the grazing intensity classification (i.e. light grazing, moderate grazing and heavy grazing) as used by the authors. This qualitative description was chosen because: 1) there was no consistency in the quantitative description of grazing given by the various authors, with different animals (e.g. sheep and cattle) and different units of measurement (e.g. sheep equivalent and percentage forage utilization) reported, and 2) we recognise that the carrying capacity of grasslands will vary depending on their climatic and geomorphic setting. In a few instances in humid areas where no clear qualitative description was given by the authors, we grouped the reported stocking densities into grazing intensities as follows: light grazing, < 5 sheep/ha; moderate grazing, 5 – 10 sheep/ha; heavy grazing, > 10 sheep/ha. We considered this classification appropriate for humid areas unlike the arid and semi-arid areas where the use of 2 sheep/ha could be considered as heavy grazing because of lower plant productivity. In some of the studies, grasslands were grazed by either cattle or yak. In such cases, we used 5 sheep units for 1 cattle and 3 sheep units for 1 yak based on equivalence values suggested by Li *et al.* (2011) and Xie and Wittig (2004). Similarly, where grazing intensity was reported only as percentage forage utilization (FU), we adopted the following classification based on the reports of Evans *et al*. (2012) and Krzic *et al.* (2014): light grazing (< 40% FU), moderate grazing (40 – 65% FU) and heavy grazing (> 65% FU).

The fertilizer studies were first grouped into N fertilizer type (ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, potassium nitrate and urea) and P fertilizer type (calcium phosphate, potassium phosphate and sodium phosphate). As with our classification of grazing intensity, we adopted qualitative descriptions of N fertilizer intensity (low N, moderate N, and high N) as used by the authors of individual studies. Where this was not indicated by the author, we grouped the intensity of N fertilizers into three N rates: low N, < 50 kg N/ha; moderate N, 50 – 150 kg N/ha; high N, > 150 kg N/ha. The lowest class boundary (50kg N/ha) was chosen to give an appropriate classification that could account for the wide range of N fertilizers (from less than 10 kg N/ha to over 600 kg N/ha) used globally. We also used the qualitative description of P fertilizer intensity given by the authors, and where this was not available, we grouped P fertilizer rates as follows: low P, < 50 kg P/ha; moderate P, 50 – 100 kg

P/ha and high P, > 100 kg P/ha. Calcium lime was used in all the liming experiments and could not be further categorised based on lime form, however, liming intensity was categorised into three rates: low lime, < 3 t/ha lime; moderate lime, 3 – 5 t/ha lime; and high lime, > 5 t/ha lime. This is based on the range of lime rates recommended for application to grasslands (e.g. DEFRA, 2011; Edmeades *et al.*, 1985).

We also categorised other factors which we thought could influence the effect of management activities on SOC. Based on the latitudes where experiments were conducted, three climatic zones were identified: Tropics (0 – 23.5°N and S), Subtropics (24 – 40°N and S), and Temperate (41 – 66°N and S). MAT in °C was divided into five categories: -5.0 to -0.1, 0.0 – 5.0, 5.1 – 10.0, 10.1 – 20.0 and 20.1 – 30. MAP was grouped into three categories: dry (< 600 mm), intermediate (600 – 1000 mm) and wet (> 1000 mm) based on previously identified global climate regimes (Dai and Wang, 2017). The duration of management activities was grouped into three: short term (< 10 years), medium term (10 – 30 years) and long term (> 30 years). The clay content was used to group soils into three textural classes: sand (< 20% clay), loam (20 – 30% clay) and clay (> 30% clay) which have been shown to be suitable for modelling large scale soil processes (Bormann, 2007).

2.2.2 Data analysis

Descriptive statistics such as minimum, maximum, mean and standard deviations were first computed for all the variables we considered, using SPSS Statistics (version 22). Subsequently, we conducted a meta-analysis based on the response-ratio approach described by Hedges *et al.* (1999) using the mixed-effect model of MetaWin software (Rosenberg *et al.*, 2000). In brief, the effect size of management activities (liming, fertilizer addition and grazing regime) on SOC stock was estimated using the natural logarithm of the response ratio (R), which is the ratio of the mean SOC stock in managed plots to mean SOC stock in control plots. i.e.

$$Effect\ size = \ln R = \ln\left(\frac{SOC\ in\ managed\ plot}{SOC\ in\ control\ plot}\right) \qquad (2.3)$$

As some of the management practices have relatively small sample sizes, the 95% confidence intervals of average effect sizes were generated through 4999 bootstrap iterations in order to overcome any violation of normality assumptions. The management effect size was considered significant (at 5% probability level) if the 95% confidence intervals did not overlap zero. A negative effect size means that management resulted in a reduction in SOC stock whereas a positive effect size implies a management-induced increase in SOC stock.

The mean effect sizes of management categories were also calculated using the approach described in the preceding paragraph. The total heterogeneity (Q_T) in each type of management practice was calculated and partitioned into within group heterogeneity (Q_W) and between group heterogeneity (Q_B). A significant Q_B (at 5% probability level) meant that management categories within that management type differed in their effects, and the exact effect of any management category was considered significantly different from that of another category when their 95% confidence intervals did not overlap. The percentage effect size of management activities was calculated from the equation:

$$Percentage\ effect\ size = (\exp(\ln R) - 1) \times 100 \qquad (2.4)$$

The effect size of management activities was further categorised according to duration of management, extent of sampling depth, clay content, climatic zone, MAT and MAP, using the categorical meta-analytic model of MetaWin software. Also, the continuous model (a weighted least square regression) of the Meta-Win software was used to analyse the linear relationships between the management effect sizes and elevation, MAT, MAP, initial SOC, clay content of the soils and duration of management. The linear model is represented as:

$$Effect\ size\ = a + b(Independent\ variable) + \mathcal{E} \qquad (2.5)$$

Where a = intercept, b = slope of the model, Ɛ = error term. The value and significance of the slope (at 5% probability level) was used to assess the influence of the independent variables on the effect of management activities on SOC stock. A negative slope indicates a greater management-induced reduction in SOC stock whereas a positive slope indicates that the negative effect of management on SOC is decreasing. Finally, we ensured that there was no publication bias (i.e. the tendency for only statistically significant results to be published by journals) by running a fail-safe test in MetaWin software.

2.3 Results

The datasets used in this study (Table 2.1) covered a wide range of climatic and elevation gradients ranging from latitudes 44°S to 65°N, longitudes 121°W to 175°E and altitudes 14 to 4800 m above sea level, with MAT that ranges from -4.8 to 26.8°C and MAP of 120 to 2000 mm. Most of the experiments were conducted in permanent grasslands with few in sown grasslands or rotated pasture (Table A2.1). Fertilizers were applied at the rates of 10 to 376 kg P/ha and 10 to 640 kg N/ha whereas 0.4 to 25.0 t/ha of lime was applied. The duration of management activities was from 0.5 to 146 years. The belowground biomass was generally higher than aboveground biomass, and both belowground biomass and aboveground biomass were slightly higher in unmanaged sites (2074 g/m^2 and 357 g/m^2) than in managed sites (2034 g/m^2 and 348 g/m^2) respectively. The grassland soils varied in texture from sandy (1.37% clay) to clayey (60% clay) and the average stock of OC within the top 2.5 to 100 cm of the soils was 40 ± 32 Mg/ha in managed grasslands and 43 ± 35 Mg/ha in unmanaged grasslands.

2.3.1 Effects of management activities on SOC stock

Our meta-analysis showed that management types and their intensity (Table 2.2 and Figure 2.2) and management duration (Table 2.3) affected SOC stock in different ways. Liming, fertilizer application and grazing resulted in an overall significant reduction (-8.5%) in SOC stock. The three management activities differed significantly ($p < 0.05$) in their separate effects on SOC stock. Grazing significantly reduced SOC stock by -15%, liming resulted in a non-significant increase (+5.8%) whereas fertilizer application significantly increased SOC stock by +6.7%. Significant variability was observed between the categories of each of these management practices. There was a progressive decline in SOC stock as the intensity of grazing increased from light (-6.9%) and moderate grazing (-13.2%) to heavy grazing (-27.1%), and the reduction in SOC stock was statistically significant at all the three levels of grazing intensity.

There was a very small and insignificant (+0.3%) effect of P fertilizer addition on soil C stock but N fertilizers significantly increased (+8.1%) SOC stock (Table 2.2). Considering the forms of N fertilizers applied, ammonium nitrate, ammonium sulphate and calcium nitrate increased SOC stock by +12.6%, +13.1% and +26.9% respectively, while there were no significant effects of ammonium chloride, urea, and potassium nitrate (Table 2.2). Low N rates resulted in a non-significant increase (+0.3%) in SOC stock whereas moderate N and high N rates significantly increased SOC by +5.2% and +13.3% respectively. The response of soil C stock to increasing lime intensity followed a completely different pattern: there were non-significant increase in SOC stock at both low

(+6.8%) and high (+2.8%) lime rates, whereas moderate lime rate led to a significant increase (+14.1%) in soil C stock (Table 2.2).

There were no significant relationships ($p > 0.05$) between the duration of liming, fertilizer addition and grazing regime, and their individual effects on SOC stock (Table 2.3). Generally, an increase in the duration of liming and fertilizer addition was associated with a greater decline in SOC stock; on the other hand, an increase in the duration of grazing leads to an increase in SOC stock. However, the overall effect of these three management activities on SOC stock was statistically significant in the short (< 10 years; -5.3%) and medium (10 – 30 years; -14.3%) term but was not significant in the long term (> 30 years; -4.5%).

2.3.2 Influence of climate and other site-specific characteristics on the response of SOC stock to grassland management

The effects of liming, fertilizer addition and grazing on SOC stock were statistically significant irrespective of elevation, clay content, the extent of soil depth sampled and the SOC contents at the start of management (Table 2.4). However, the modifying effect of clay on management activity was only significant in fertilized grasslands, with increasing clay content resulting in a greater reduction in SOC stock ($b = -0.0008$, $p = 0.042$) (Table 2.4).

The overall management-induced reductions in SOC stock were significant across all climatic zones in the order: tropics > subtropics > temperate, and across all MAT classes with effect size increasing with increasing temperature class above 0°C (Table 2.5). There were statistically significant relationships between MAP, MAT and the effects of management practices on SOC stock. The smallest effect of management on SOC stock was found when MAT was in the range of 0 – 5.0°C (-4.9%). Management effects on SOC stock were greater at MAT below 0°C (-8.4%) and above 5.0°C (-17.2%). The management effect was only significant when MAP was below 600 mm (-11.7%; Table 2.5).

On further analysis, it was only in the temperate zone that the relationships between MAT, MAP and the effects of management activities on SOC stock were statistically significant. Within the temperate zone, the overall negative effect of management practices decreased with increasing MAT ($b = 0.0108$, $p = 0.00054$, $n = 195$) and MAP ($b = 0.0002$, $p = 0.0000$, $n = 195$) (Table 2.5). When management activities were considered individually, the effects of grazing and N fertilizer application exhibited a positive relationship with MAT and MAP, whereas the negative effect of liming decreased with increasing MAT and increased with increasing MAP (Table 2.6; Figure 2.3). When the relationships between management and temperate zone climatic parameters were explored by management intensity, the MAP-grazing relationship was only significant for heavy grazing (b = 0.0007, p = 0.00063), the MAP-N fertiliser relationship was only significant for low N fertilisation (b = 0.0004, p = 0.01167), the MAT-N fertiliser relationship was only significant for moderate N fertiliser (b = 0.0082, p = 0.04342), and MAP-lime (b = -0.0001, p = 0.02246) and MAT-lime (b = 0.0198, p = 0.00885) relationships were only significant for low lime categories (Table 2.6).

Table 2.1: Characteristics of datasets used for meta-analysis. n = number of datasets.

Variable	n	Minimum	Maximum	Mean	Standard deviation
SOC of managed sites (Mg/ha)	341	0.93	204.12	40.14	32.30
SOC of control sites (Mg/ha)	341	1.80	200.81	43.39	35.35
Aboveground biomass of managed sites (g/m^2)	131	7.20	7998.00	348.43	988.55
Aboveground biomass of control sites (g/m^2)	131	7.40	6225.00	356.75	686.30
Belowground biomass of managed sites (g/m^2)	50	27.70	32487.00	2033.77	4650.11
Belowground biomass of control sites (g/m^2)	50	82.50	26188.00	2074.02	4111.07
Initial SOC of sites (%)	35	0.19	17.40	4.46	4.42
Clay content of study sites (%)	98	1.37	60.00	19.57	13.60
Elevation (m)	341	14.00	4800.00	1619	1324
Latitude (^{o})	341	-44.00	65.04	31.13	26.34
Longitude (^{o})	341	-121.75	175.75	45.36	91.90
Mean annual temperature (˚C)	341	-4.80	26.80	5.60	6.60
Mean annual precipitation (mm)	341	120.00	2000.00	594.00	377.00
Duration of management (years)	341	0.50	146.00	18.97	22.25
Soil depth (cm)	341	2.50	100.00	14.70	12.10
Calcium lime (t/ha)	20	0.40	25.00	7.44	8.32
Nitrogen fertilizer rate (kg N/ha)	71	10.00	640.00	137.41	129.30
Phosphorus fertilizer rate (kg N/ha)	18	10.00	376.00	83.08	88.64

Table 2.2: Effect of management (mgt) activities on SOC stock. * = significant, ns = not significant at 5% probability level, n = number of data.

Management type	Management category	Effect on SOC stock (%)	Management intensity	Effect on SOC stock (%)
All mgt type (n = 341)		-8.5*		
Grazing (n = 232)		-15.0*	Light grazing (n = 100)	-6.9*
			Moderate grazing (n = 67)	-13.2*
			Heavy grazing (n = 65)	-27.1*
Liming (n = 20)		+5.8^{ns}	Low lime (n = 8)	+6.8^{ns}
			Moderate lime (n = 5)	+14.1*
Fertilization (n =89)		+6.7*	High lime (n = 7)	+2.8^{ns}
	All nitrogen fertilizer (n = 71)	+8.1*		
	Ammonium nitrate (n = 28)	+12.6*	Low nitrogen (n = 19)	+0.3^{ns}
	Ammonium chloride (n = 3)	-5.4^{ns}	Moderate nitrogen (n = 29)	+5.2*
	Ammonium sulphate (n = 9)	+13.1*	High nitrogen (n = 23)	+13.3*
	Urea (n = 24)	+3.6^{ns}		
	Potassium nitrate (n = 4)	-1.0^{ns}		
	Calcium nitrate (n = 3)	+26.9*		
	All phosphorus fertilizer (n = 18)	+0.3^{ns}	Low phosphorus (n = 8)	-2.1^{ns}
	Calcium phosphate (n = 10)	-5.0^{ns}	Moderate phosphorus (n = 6)	+5.6^{ns}
	Potassium phosphate (n = 5)	+7.5*	High phosphorus (n = 4)	+1.7^{ns}
	Sodium phosphate (n = 3)	-7.8^{ns}		

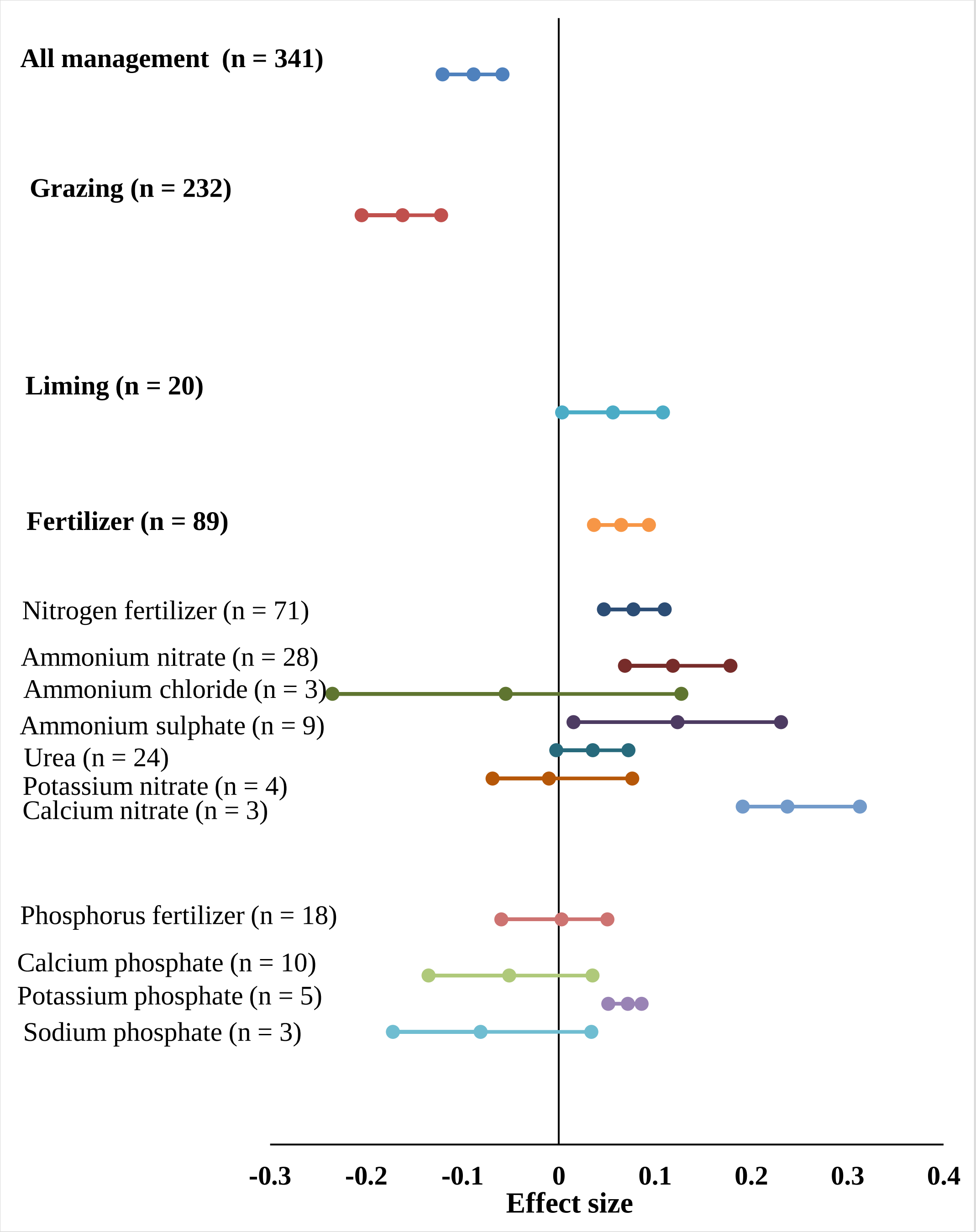

Figure 2.2: Effect sizes of fertilizer application, liming and grazing on SOC stock (bars represent mean plus and minus 95% confidence intervals).

Table 2.3: Influence of management duration on the response of SOC stock to fertilizer, liming and grazing. * = significant, ns = not significant at 5% probability level, n = number of datasets, a = intercept, b = slope, p-value^S = p-value for the regression slope.

Management duration (years)	Range (years)	Management effect on SOC (%)	Effect size		
			a	b	p-value^S
< 10 years (n = 164)		-5.3*			
10 – 30 years (n = 124)		-14.3*			
> 30 years (n = 53)		-4.5^{ns}			
Grazing duration (n = 232)	1 – 91		-0.1731	0.0006	0.37041
Liming duration (n = 20)	0.5 – 73		0.0877	-0.0007	0.57725
Fertilizer duration (n = 89)	3 – 146		0.0567	-0.0002	0.62739
All management duration (n = 341)	1 – 146		-0.0960	0.0004	0.48688

Table 2.4: Overall effect of fertilizer, liming and grazing on SOC stock under different site characteristics. * = significant, ns = not significant at 5% probability level, n = number of datasets, I = initial, a = intercept, b = slope, p-value^S = p-value for the regression slope.

Factor	Factor category	Range	Effect on SOC (%)	Effect size		
				a	b	p-value^S
Depth extent	0 – 19 cm (n = 248)		-6.6*			
	0 – 40 cm (n = 85)		-11.9*			
	0 – 100 cm (n = 8)		-24.0*			
Clay content	< 20% Clay (n = 57)		-11.9*			
	20 to 30% Clay (n = 27)		-11.8*			
	> 30% Clay (n = 14)		-16.9*			
	%Clay in grazed site (n = 70)	1.4 – 60		-0.2477	0.0025	0.22596
	%Clay in limed site (n = 5)	29 – 29		-0.0254	-0.0005	1.00000
	%Clay in fertilized site (n = 23)	4.3 – 23		0.1135	-0.0088*	0.04248
Elevation	Elevation (m) (n = 341)	14 – 4800		-0.1007	0.0000	0.38066
ISOC	ISOC of grazed site (%)(n = 18)	0.2 – 7.0		-0.1241	-0.0029	0.86654
	ISOC of limed site (%) (n = 4)	6.4 – 17.4		0.1584	-0.0087	0.62526
	ISOC of fertilized site (%) (n = 13)	0.2 – 8.6		0.0642	-0.0153	0.47418

Table 2.5: Overall effect of fertilizer, liming and grazing on SOC stock under different climate conditions. MA = mean annual, T = temperature, P = precipitation, Mgt = management, n = number of datasets, bold values are significant at 5% probability level, italicized value is the largest slope, a = intercept, b = slope, p-value^S = p-value for the slope.

Factor	Factor category	Range	Mgt effect on SOC stock (%)	Effect size		
				a	b	p-value^S
Climatic zone	Tropics (n = 24)		**-22.4**			
	Subtropics (n = 122)		**-12.5**			
	Temperate (n = 195)		**-4.5**			
MAT	-5.0 to -0.1°C (n = 55)		**-8.4**			
	0 to 5.0°C (n = 138)		**-4.9**			
	5.1 to 10.0°C (n = 84)		**-10.2**			
	10.1 to 20.0°C (n = 48)		**-12.8**			
	20.1 to 30.0°C (n = 16)		**-17.2**			
MAP	< 600 mm (n = 223)		**-11.7**			
	600 to 1000 mm (n = 66)		0.4			
	> 1000 mm (n = 52)		-5.2			
MAT	MAT (°C) (n = 341)	-4.8 – 26.8		-0.0581	**-0.0053**	0.00465
	MAT tropics (°C) (n = 24)	4 – 26.8		-0.4056	0.0074	0.40919
	MAT subtropics (°C) (n = 122)	-4.3 – 19.0		-0.0753	**-0.0092**	0.00008
	MAT temperate (°C) (n = 195)	-4.8 – 15.5		-0.0878	***0.0108***	0.00054

Table 2.5 (continued): Overall effect of fertilizer, liming and grazing on SOC stock under different climate conditions. MA = mean annual, T = temperature, P = precipitation, Mgt = management, n = number of datasets, bold values are significant at 5% probability level, italicized value is the largest slope, a = intercept, b = slope, p-value^S = p-value for the slope.

Factor	Factor category	Range	Mgt effect on SOC stock (%)	Effect size		
				a	b	p-value^S
MAP	MAP (mm) (n = 341)	120 – 2000		-0.1387	**0.0001**	0.01079
	MAP tropics (mm) (n = 24)	520 – 1230		-0.5338	0.0004	0.07645
	MAP subtropics (mm) (n = 122)	120 – 1850		-0.1332	0.0000	0.91539
	MAP temperate (mm) (n = 195)	120 – 2000		-0.1362	**0.0002**	0.00000

Table 2.6: The relationship between climatic variables in the temperate zone and the effect sizes of management intensity on SOC stock. Bold values are significant at 5% probability level, n = number of datasets, a = intercept, b = slope, p-value^{S} = p-value for the slope.

Management effect size	Temperate MAT				Temperate MAP			
	n	a	b	p-value^{S}	n	a	b	p-value^{S}
Grazing	113	-0.1702	0.0057	0.32638	113	-0.2511	**0.0002**	0.00219
Light grazing	50	-0.0709	-0.0062	0.52051	50	-0.1291	0.0001	0.36422
Moderate grazing	39	-0.1591	0.0136	0.12257	39	-0.2135	0.0002	0.05223
Heavy grazing	24	-0.4712	0.0250	0.13653	24	-0.7038	**0.0007**	0.00063
Liming	14	-0.0709	**0.0235**	0.00196	14	0.2492	**-0.0001**	0.01021
Low lime	7	-0.0543	**0.0198**	0.00885	7	0.2273	**-0.0001**	0.02246
Moderate lime	4	-1.6644	0.1869	0.45149	4	0.2451	-0.0001	0.51269
High lime	3	-1.5918	0.1792	0.06106	3	0.2756	-0.0002	0.86640

Table 2.6 (continued): The relationship between climatic variables in the temperate zone and the effect sizes of management intensity on SOC stock. Bold values are significant at 5% probability level, n = number of datasets, a = intercept, b = slope, p-value^S = p-value for the slope.

Management effect size	Temperate MAT				Temperate MAP			
	n	a	b	p-value^S	n	a	b	p-value^S
Nitrogen fertilizer	56	0.0468	**0.0107**	0.00356	56	0.0170	**0.0001**	0.04711
Low nitrogen	10	0.0059	0.0130	0.15058	10	-0.1680	**0.0004**	0.01167
Moderate Nitrogen	23	0.0261	**0.0082**	0.04342	23	0.0112	0.0001	0.20137
High nitrogen	23	0.0847	0.0132	0.15513	23	0.0699	0.0001	0.36717

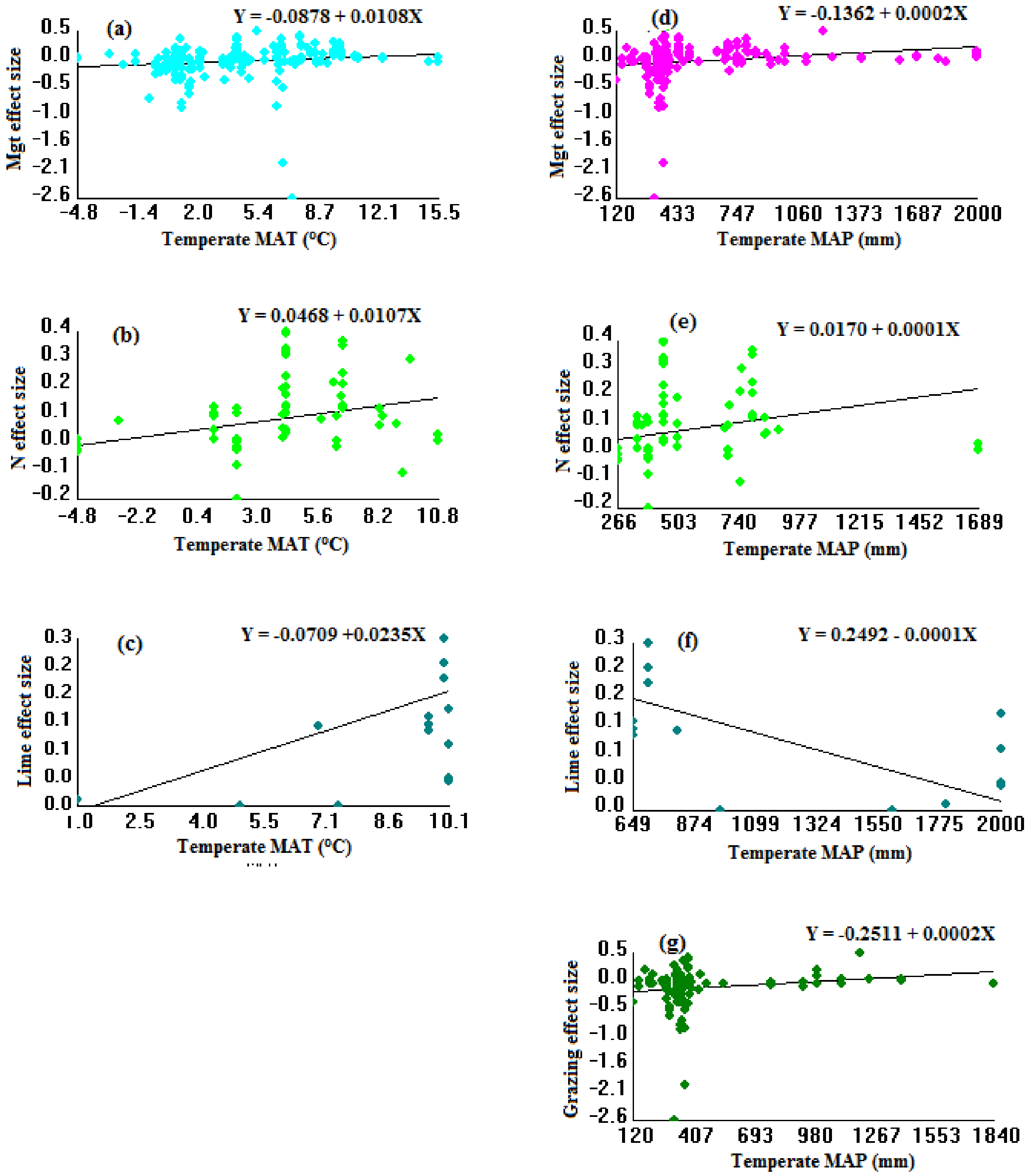

Figure 2.3: Statistically significant relationships between temperate MAT and the effect size of all management (a), nitrogen addition (b), and lime (c) on SOC stock; and between temperate MAP and the effect size of all management (d), nitrogen addition (e), lime (f) and grazing (g) on SOC stock. The interactive effect of grazing effect size and MAT was not significant and was therefore excluded in the figure.

2.4 Discussion

This study reveals an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, mainly attributable to grazing (-15.0%), and only partially attenuated by fertilizer addition (+6.7%) and liming (+5.8%), indicating that management to improve biomass production does not contribute sufficient organic matter to replace that lost by direct removal by animals. Management activities had the greatest effect in the tropical zone (-22.4%) and in zones with low MAP (-11.7%), suggesting a sensitivity at extremes of the climate envelope. The large effect in the tropical zone likely reflects the drastic change in plant biomass inputs to soil when forests are converted to grasslands. In the temperate zone the overall size of the negative management effect was small (-4.5%), but was positively related to MAT and MAP, indicating that soil C stocks may be relatively robust to management under anticipated future regimes of climate warming. Our two hypotheses that grassland management practices will result in SOC decline and that this effect will be influenced by climatic settings were therefore confirmed.

2.4.1 The net effect of liming, fertilizer addition and grazing on the stock of C in grassland soils

This study showed that grazing had an overriding effect on grassland SOC stock. The significant reductions in SOC stock resulting from grazing (-15%) is larger but consistent with the results of previous published meta-analyses (e.g. -9%, Dlamini *et al.*, 2016; -10%, Zhou *et al.*, 2016). In our study, the negative effect of grazing doubles as the intensity of grazing increased from light to moderate grazing, and from moderate to heavy grazing. Heavy grazing therefore resulted in the most significant reduction in SOC stock (-27.1%) and this can primarily be attributed to excessive removal of vegetation and consequently limited litter returns to the soil. Hans *et al.* (2008) reported a 74% forage utilization under heavy grazing, with the amount of litter returns being only 45% of the values in lightly grazed sites. This happens because a significant proportion of grassland vegetation fed to livestock is subsequently lost from the ecosystem through animal respiration, methane expulsion and export of products such as milk (Soussana *et al.*, 2007). There is also the tendency for plant meristems to be removed under heavy grazing, leading to a reduction in plant growth capacity (Conant and Paustian, 2002; Mestdagh *et al.*, 2006). Of the plant C ingested by animals, only about 25-40% is returned to the grassland as excreta (Soussana *et al.*, 2007). Even this relatively small amount of C that is returned to the soil as animal excreta does not always imply an increase in soil C because the excreta contains readily utilizable substrates that stimulate soil microbial activities in grasslands (Clegg, 2006), leading to a greater decomposition of organic materials and soil C loss (Grayston *et al.,* 2004). This was demonstrated when livestock was excluded for seven years from a grassland in northern England, which resulted in a 20% reduction in the activity of soil microorganisms (Medina-Roldan *et al.,* 2012). In addition, Ritz *et al.* (2004) found a higher microbial biomass due to increasing sheep urine patches and Williams *et al.* (2000) reported an increase in active bacteria number relative to fungi with the addition of synthetic sheep urine.

The removal of standing plants and litter also exposes the soil to erosive precipitation and wind thereby accelerating erosion and C loss (Steffens *et al.,* 2008; Tanentzap and Coomes, 2012; Xie and Wittig, 2004). For example, Han *et al.* (2008) found that only 33-36% of the ground was covered by vegetation after two years of heavy grazing. Heavy grazing may also cause soil C loss through trampling and poaching (Ma *et al.*, 2016). Trampling from grazing animals results in soil compaction, characterised by increased bulk density and reduced infiltration (Marshall *et al.,* 2014, 2009). This may increase runoff events and export particulate soil C to surface waters (Meyles *et al.*, 2006; Robroek *et al.,* 2010). It has also been reported that the rate of photosynthesis decreases significantly under simulated sheep trampling (Clay and Worrall, 2013). This reduces plant productivity and potential amount of C inputs to the soil.

The practice of improving grassland productivity by applying high rates of lime and N fertilizer is likely not to increase soil C storage under heavy grazing regimes. In this study, SOC stock declined from 14.1% at moderate liming (3 – 5 t/ha) to 2.8% at high liming rate (>5 t/ha). Wang *et al.* (2016) also found that increasing the rate of liming (e.g. by 12.5 t ha^{-1}) led to a decrease of about 14% in SOC at the surface (0-10 cm) soils of some pastures in Australia. At high lime rates, soil acidity-related constraints are removed (Orgill *et al.,* 2015) and this leads to an increase in microbial respiration, a faster decomposition of organic materials and an increase in the level of DOC in soil solution, which is prone to leaching and erosion losses (Hornung *et al.,* 1986; Mijangos *et al.,* 2010; Staddon *et al.,* 2003). In addition, if root growth is stimulated by liming, there is an increase in the release of exudates from grass species at higher soil pH levels which acts as a primer to enhance SOM decomposition and C loss (Grayston *et al.*, 2004). Generally, the potential contribution of liming to global grassland SOC stock gain is limited because the practice is confined to acidic soils which are mostly found in sites that are heavily leached by precipitation (i.e. high MAP). This can be seen in this study because relative to all the management activities we considered, liming studies were very few and the bulk of the papers we used were from low MAP areas, reflecting the climatic zone in which grasslands naturally occur.

At high rates of N fertilizer application, additional SOC stock gained by grassland soils for every unit of N fertilizer added has been shown to decline (i.e. a reduction in C gain efficiency at high rates of N fertilizer addition). Amman *et al*. (2009) found that the application of 230 kg N ha^{-1} $year^{-1}$ for six years in a Swiss grassland caused only about 6% increase in soil C gain, compared to the 13% gain at high N rate (>150 kg N/ha) in our study. Fornara and Timan (2012) studied the effect of 27 years of N addition (ranging from 0 to 270 kg N/ha) on C sequestration in prairie grassland soils in Minnesota, USA, and found that SOC stock increased with increasing N rates but net C gain per unit of added N significantly decreased after 10 kg N/ha. Also, after six years of subjecting some grasslands in Northern China to six levels of N addition (ranging from 0 to 560 kg N/ha), He *et al*. (2013) reported an increase in SOC stock from 118 to 131 t/ha within the surface 0-100 cm of the soil, but there was a decreasing C gain efficiency as added N increased. These findings are comparable with our study because we found a greater decline in soil C stock as the duration of fertilization increased, however, this negative effect peaks in the medium term (10 – 30 years) and becomes insignificant afterwards. Overall, there is evidence that the positive effect of high N addition declines over time and may also increase the risk of emissions of other more potent greenhouse gases such as nitrous oxide (N_2O) into the atmosphere beyond background levels (Jarvis *et al*., 2001; Vuichard *et al*., 2007), thereby negating any C sequestration benefits. Our study focused only on the potential for long term C accrual in managed grassland soils rather than net greenhouse gas emissions, and there is a need for future studies to synthesize the net effect of management activities on the balance of greenhouse gases. This will provide a clearer picture of the full implication of grassland management to climate change.

This study indicates that intensive grazing-related grassland management activities (particularly liming and N fertilization) and heavy grazing are not a sustainable management regime. Management intensification depletes SOC stock potentially increasing the atmospheric CO_2 concentration and exacerbating the already climate warming trajectory. Future grassland management policy particularly in the tropics with the greatest management-induced decline in SOC stock, should therefore focus mainly on reducing the intensity of grazing. This can be best achieved by excluding grasslands from grazing (Xiong *et al.*, 2016). However, since there is need to balance the goal of soil C sequestration with the need for livestock production, grasslands should be maintained under moderate grazing regimes and governments should consider setting up environmental schemes as an incentive to encourage farmers to adopt less intensive management activities.

2.4.2 Influence of climate on the response of SOC stock to liming, fertilizer application and grazing

This study revealed that climate significantly influenced the overall effect of liming, fertilizer application and grazing on SOC stock. This is in line with reports in previous studies (e.g. Chimner and Welker, 2011; McSherry and Ritchie, 2013; Zhou *et al.*, 2016) which showed that climate exerts significant influence on the effects of management on grassland C cycling. In our study, the temperate zone had the smallest management-induced decline in SOC stock (-4.5%) and yet exhibited a greater interactive effect of climate compared to either the tropics or the sub-tropics. Negative effects of management declined significantly with increasing MAT and MAP which is a strong indication that increasing temperature and precipitation in temperate grasslands has the potential to reverse the overall management-induced decline in SOC stock of these areas and possibly increase C sequestration. The strong positive temperature-management interactive effects on SOC stock of temperate grasslands can be explained by temperature-induced increase in the length of growing season. Increasing temperature extends the length of the growing season in temperate environments (Hunt *et al.* 1991) thereby enhancing plant growth and C additions to the soil (Chang *et al.*, 2016). For example, relative to 1961-1990 average of 252 days, the length of growing season in England increased to 282 days in 2012 (DECC, 2013) in response to about 1.7°C increase in MAT (Jenkins *et al.* 2008). Xia *et al.* (2014) observed that increasing spring temperature at latitudes between 30 and 90°N stimulates the onset of leaf unfolding which results in an earlier start of the growing season and enhances ecosystem productivity.

The positive response of SOC stock to increasing temperate and precipitation in the temperate zone may also result from greater nutrient availability and biomass production due to removal of restrictions on mineralization imposed by cold and dry conditions. In dry (MAP <600mm) and/or cold conditions (MAT<0°C), characteristics of many temperate grasslands, there is lower biomass production which limits litter inputs to the soil leading to low C stock (De Deyn *et al.*, 2008; Garcia-Pausas *et al.*, 2007). As the climate becomes warmer and wetter, increased mineralisation of organic materials by soil microbes increases nutrients available for plants' uptake thereby increasing grassland biomass production (Davidson, 2015; Guo and Gifford, 2002; Xiong *et al.*, 2016) and litter returns to soil. High temperatures (e.g. > 20°C) stimulates higher soil microbial activities such as respiration and OM decomposition, which results in the loss of soil C as CO_2 or CH_4 into the atmosphere (Ward *et al.*, 2013) or as DOC in soil solution. However, it is not likely that increased warming of the temperate zone in the 21st Century (IPCC, 2013) will stimulate higher C loss via soil microbial respiration compared to enhanced biomass production resulting from warmer and wetter climate and grassland improvement activities such as liming and N fertilization.

Thus, temperate grasslands will potentially serve as a C sink in the face of climate change due to increasing temperature. This will contribute significantly to global climate regulation because temperate grasslands are widely distributed in most continents (Dixon *et al.*, 2014) e.g. the Pampas of South America, the Plains and Prairies of North America, the Steppes of Eurasia, the Downs of Australia and New Zealand, and the Veldts of Africa. However, since the climate change trajectory in temperate zones is an all-season increase in temperature, an increase in winter precipitation and a decrease in summer precipitation (Jenkins *et al.*, 2008), there is still a possibility for the legacy effects of high rate of evapotranspiration in summer to negate effects of increased precipitation in winter, which may lead to drier conditions and enhance management-induced soil C loss.

Therefore, in order to ensure sustainable provision of various ecosystem services by temperate grasslands particularly forage for livestock and climate change mitigation via carbon sequestration, there is need to further study how projected changes in climatic conditions (e.g. warming, drought and wetter conditions) will influence SOC storage and fluxes. A number of manipulative experiments have already been conducted in temperate grasslands to study the effects of climate change on the ecosystem, and involved the use of regulated heating to simulate desired temperature increase, with either an addition or exclusion of water to simulate wet or drought condition. The results of these climate experiments in temperate grasslands were synthesised by White *et al.* (2012) but they found a mixed and complex results with no consistent pattern of grassland response. White *et al.* (2012) concluded that climate change effects on temperate grasslands remain poorly understood and this underscores the need for further research. As temperate grasslands are subjected to different management regimes, it is necessary to conduct more site specific experiments that consider the interactive effects of climate change and grassland management activities such as fertilizer application and liming under different grazing regimes. This will provide an improved understanding of mechanisms operating at each of the global regions of temperate grasslands, and help inform appropriate policy decisions.

2.5 Conclusion

There was an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands, due primarily to grazing (-15%), which was partly weakened by fertilizer addition (+6.7%) and liming (+5.8%). This indicated that grazing-related management activities to improve biomass production does not contribute sufficient soil C to replace the C loss via animal grazing. SOC loss was greatest in the tropics and mainly under heavy grazing, and we recommend that future grassland management policy should focus on reducing the intensity of grazing. Temperate grasslands had the least management-induced decline in SOC stock but it was positively related to MAT and MAP such that increasing MAT and MAP reduced the negative management effects. This was an indication that temperate grasslands are potential C sinks in the face of climate change. However, the understanding of the mechanisms of interactions between climate change and management activities in temperate grasslands is still poor. Therefore, in order to ensure a sustainable management of grasslands that will provide adequate forage for livestock and mitigate climate change through C sequestration, we recommend further studies looking at the interactive effects of projected climate change and management regimes on soil C stock.

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Chapter 3: Soil organic carbon stock and fractional distribution in upland grasslands

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Abstract

Upland grassland soils are an important terrestrial C store and provide vital ecosystem services such as climate regulation. The C stocks in these soils are subject to changes due to management activities. In this study, we compared SOC stocks and fractions under traditional hay meadow and conventional (silage or permanent) pasture management regimes at two upland grassland locations within the Yorkshire Dales, northern England, United Kingdom. Stocks of SOC in the top 15 cm of the grassland soils were determined from bulk soil samples and in soil fractions after a combination of physical and chemical fractionation. Results showed that these upland grasslands stored large amounts of organic C in the top 15 cm of their soils, ranging from 58.93±3.50 to 100.69±8.64 Mg ha^{-1}. Overall, there was little contrast between the sites except that soil C stock was significantly higher under the Nidderdale silage pasture that received the highest N inputs, possibly due to enhanced vegetation growth and subsequent litter return to the soil. No effect of soil parent material was observed on soil C stock due to liming-induced increase in the pH of Nidderdale soils and neither management activities nor soil parent materials affected the distribution of soil C into different fractions. In all sites, about 70% of the SOC stock was protected in stable soil fractions, specifically in the mineral soil mass, indicating a potential for the grasslands to contribute in climate change mitigation. However, due to the rapid turnover of the labile C pool and especially in a changing climate, it is recommended that further research be carried out in order to understand the turnover rates of labile C pools and whether management activities alter the distribution between the decomposition products. This will help to better understand whether rapid turnover of labile C will negate the likely benefits of stable C pools to climate change mitigation.

Keywords: Soil carbon dynamics; carbon fractions; grassland management; upland soils; soil carbon stock

3.1 Introduction

Grasslands store a large amount (~90%) of its sequestered C in the soil (Ajtay *et al.,* 1979), and often have higher soil C stock relative to other vegetation types in a given climate regime (IPCC, 2003). Management of grasslands can range from extensive practices such as no/minimal chemical inputs and occasional grazing to intensive practices such as regular chemical additions including lime, N, P and K containing fertilizers, and year-round grazing (Tiemeyer *et al.,* 2016). Generally, these management activities have been shown to influence soil C dynamics (Soussana *et al.,* 2007) but the exact effects of each management practice on C stock are not fully understood. For example, variable results have been reported in a number of studies that investigated how soil C respond to different management activities such as grazing intensity (Burrows *et al.,* 2012; Han *et al.,* 2008; Schuman *et al.,* 1999), liming (Mijangos *et al.,* 2010; Sochorova *et al.,* 2016; Wang *et al.,* 2016), and fertilizer application (Fornara *et al.,* 2013; He *et al.,* 2013; Neff *et al.,* 2002). These studies reported either an increase, a decrease or no change in soil C stock due to management activities. The inconsistencies in these results may be due to differences in soil types, specific management activities and duration of management regimes as they change over time, for example, in response to financial incentives such as agri-environmental schemes.

An important area of current research interest is understanding the factors controlling soil C stock and its stability in response to grassland management (e.g. Ward *et al.,* 2016). This is because storage of soil C in long-term pools is necessary for climate change mitigation and requires stabilization in forms that are less susceptible to loss (Adkins *et al.,* 2016; Lal and Kimble, 1997; O'Brien and Jastrow, 2013). The stabilization mechanisms may be through: 1) biochemical formation of organic C compounds with molecular structures that make them relatively more resistant to decomposition, 2) interaction of organic C with soil mineral particles particularly clay and silt, and 3) occlusion of organic C within soil aggregates (Breulmann *et al.,* 2016; Sollins *et al.,* 1996). In recent years, the idea of SOC possessing recalcitrant properties which make it stable in the soil has been challenged (Amelung *et al.*, 2008; Lehman and Kleber, 2015; Schmidt *et al.*, 2011) and the key mechanism of stabilization now favoured by researchers is physical protection of SOC from microbes regardless of its chemical structure (Dungait *et al.*, 2012; Kleber *et al.*, 2011). Also, the most important physical mechanism for stabilizing soil C is its association with reactive soil mineral particles because access by microbes and enzymes is limited (Conant *et al.*, 2011; Mikutta *et al.*, 2006). This has recently been corroborated by the results of a meta-analysis of 376 published laboratory incubation data, where clay content was found to be the most crucial regulator of SOC decomposition (Xu *et al.*, 2016).

SOC is usually fractionated into functional pools such as labile (or active) and stable (or passive) pools, with different turnover rates or mean residence times (ranging from days to centuries and millennia), and this often serves as a relative measure of stability (Breulmann *et al.*, 2016; Cambardella and Elliot, 1992; Roscoe and Machado, 2002; Von Lutzow *et al.*, 2007). The active C pools such as POC, DOC and MBC have the fastest turnover and are more sensitive to land management than the stable pools (Alvarez and Alvarez, 2016; deFigueiredo *et al.*, 2010; Van Leeuwen *et al.*, 2015). It is therefore important to study not just the changes in total SOC in response to environmental change, which requires long term monitoring to be able to detect, but the partitioning and dynamics of the various soil C pools. This would improve understanding of land management effects on SOC even in the short term.

In the UK, the greater number of studies on the response of soil C to management activities have focused on lowland grasslands (e.g. Clegg, 2006; Clegg *et al.*, 2003; Hopkins *et al.*, 2009, 2011), with results showing either a decrease, an increase or no change in soil C stock. However, a recent report based on a study of 180 permanent grasslands (mostly lowlands) in the UK, revealed a decrease in soil C stock with increasing management intensity (Ward *et al.*, 2016). This study also suggested that soil parent material might have influenced the response of soil C to management activities (Ward *et al.*, 2016) but this has not fully been investigated for UK grasslands. The effect of soil parent material is possible because there is a likely interaction between inherent soil characteristics and management activities that subsequently influences soil C dynamics. For example, in northern Spain, Mijangos *et al.* (2010) studied the effects of liming on soil characteristics and plant productivity in calcareous and siliceous grasslands; they found that liming significantly increased soil pH and microbial activity in the siliceous grassland compared to the calcareous grassland. Soil acidity is important for C dynamics because C tends to accumulate in the soil under acidic conditions, due to the predominance of fungi with high biomass and restricted microbial decomposition of organic materials (Alexander, 1977; Bardgett *et al.*, 1993). This is also evident in the report of the UK Countryside Survey of 2007, where acid grassland soils store 82 t ha^{-1} C compared to neutral grassland soils which contain 61 t ha^{-1} C within 15 cm of the soil surface (Carey *et al.*, 2008). Leifeld *et al.* (2013) also found that a one pH unit of acidification resulted in 22 to 86% increase in mean residence times of organic C pools.

Acidic soil conditions prevail in the UK's upland grasslands (grasslands mostly above 300 m altitude) primarily due to base-poor geology and high leaching associated with cool and wet climate (Floate, 1977; Holden *et al.*, 2006). Elevated C stocks in these wet, acidic upland soils is therefore to be expected. Yet, to the best of our knowledge, the dynamics of SOC in the mineral soils of the British upland grasslands remains poorly understood. These upland grasslands are mostly used for livestock production (Leifeld and Fuhrer, 2009; Medina-Roldan *et al.*, 2012; Stevens *et al.*, 2008) under management options that typically are influenced by environmental stewardship schemes, which aim to protect and enhance biodiversity. The interaction between parent material and management activity on soil C stock and stability has received relatively little research attention, and yet there are potentially important implications for development of new agri-environmental stewardship schemes and climate mitigation strategies. Therefore, the aim of this research is to assess the stock and fractions of SOC in the mineral soils of upland grasslands in northern England under: 1) traditional hay meadow and conventional pasture management regimes, and 2) soils of siliceous stone and limestone parent materials. We hypothesize that 1) SOC stock will be higher in soils under traditional hay meadow with intermittent grazing compared to soils under conventional pasture, 2) SOC stock will be lower in soils developed on limestone compared to soils that are poorly drained and developed on base-poor parent material due to likely effects of acidity and higher moisture content on organic matter decomposition, and 3) greater proportions of SOC stocks will be protected in stable forms due to high mineral contents of the soils at all the locations investigated.

3.2 Methodology

3.2.1 Study area

Two locations within the Yorkshire Dales, an upland area of the Pennines in Northern England, UK, were selected for this study; one in Nidderdale (54°09'N, 01°53'W) and the other in Ribblesdale (54°05'N, 02°16'W). Both locations are at an elevation of approximately 300 m and a distance of about 20 km apart. These two locations were chosen because of their contrasting soil parent material (siliceous stones and limestone, respectively), which we expected to affect soil properties differently and how the soils would respond to management activities. The two locations are characterized by shallow soils, Stagnohumic gley at Nidderdale and Brown earth at Ribblesdale, with maximum depth to compacted layer being 20 cm. Mean annual temperature is 7.4°C and mean annual rainfall is 1550 mm, based on 1981 to 2010 average. At each location, there are two contrasting grassland management regimes: traditional hay meadow managed under an agri-environment scheme and conventional pasture managed for silage (Nidderdale) or permanent grass (Ribblesdale), making a total of four sites. The traditional hay meadows under agri-environment schemes are managed with the aim of restoring, protecting and enhancing biodiversity. At the time of this study, hay meadow management typically involved avoiding inputs of inorganic fertilizer, no cutting before July, no grazing during spring and early summer, and re-seeding with a wildflower mix where necessary (Natural England, 2010). Hay meadow management was very similar at the two locations. In contrast to the hay meadows, the management regime of conventional pasture was quite different at the two locations, and at the Nidderdale site was very similar to the hay meadow except that the field managed for silage received inorganic N and additional inputs of P and K in poultry manure, and had recently been reseeded with ryegrass (Table 3.1). At the Ribblesdale location, the permanent pasture received no organic manure inputs or fertilizer and was not cut, but was continuously grazed by sheep (Table 3.1). The Nidderdale site had been limed to raise soil pH three years before sampling.

Table 3.1: Description of the study area.

	Site and Management Information			
Site	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
Location	54°09'52"N, 01°53'17"W	54°09'50"N, 01°53'22"W	54°05'34"N, 02°15'55"W	54°05'35"N, 02°16'1"W
Elevation (m)	300	302	300	301
Geology	Clay drift with siliceous stone	Clay drift with siliceous stone	Carboniferous limestone	Carboniferous limestone
Soils	Stagnohumic gley	Stagnohumic gley	Brown earth	Brown earth
Dominant vegetation	*Holcus lanatus, Lolium perenne, Ranunculus repens, Trifolium repens*	*Lolium perenne, Holcus lanatus, Ranunculus repens*	*Anthoxanthum odoratum-Geranium sylvaticum* grassland	*Holcus lanatus, Poa trivialis, Festuca rubra, Deschampsia cespitosa, Alopecurus pratensis, Lolium perenne*
Management regime	Traditional hay meadow under an agri-environment scheme	Conventional pasture	Traditional hay meadow under an agri-environment scheme	Conventional pasture
Management history	Traditional hay meadow for over 150 years	Traditional hay meadow until about 1960, used as rough pasture between 1960 and 2008, utilized for silage since 2008	Traditional hay meadow for over 25 years	Conventional pasture for over 25 years
Re-seeding	Wild flowers over last six years	Perennial ryegrass broadcast in July 2015	none	none

Inorganic fertilizer application	none	Surface application of 50 kg N ha^{-1} $year^{-1}$ since 2014 as NH_4NO_3 or $(NH_4)_2SO_4$ in early June	None since 1995. Annual dressing of 20 kg N ha^{-1} as NPK 20:10:10 from 1980 to 1995	None
Organic manure application	Surface application of 6 t ha^{-1} $year^{-1}$ of FYM in May.	Surface application of 6 t ha^{-1} $year^{-1}$ of FYM in May. 100 kg each of both phosphate and potash applied in early June 2016 as burnt poultry manure	Surface application of 4-6 t ha^{-1} $year^{-1}$ of FYM from 1990 to 2001	None
Liming	Surface application of 5 t ha^{-1} Mg limestone in 2010 and 5 t ha^{-1} Ca limestone in 2013	Surface application of 5 t ha^{-1} Mg limestone in 2010 and 5 t ha^{-1} Ca limestone in 2013	None since 1995. There was occasional annual dressing previously	none
Livestock stocking rate/grazing intensity	10-15 sheep ha^{-1} in the month of April and from September to the end of December. Higher stocking density in September when the field is used as a holding area	15-20 sheep ha^{-1} in the month of April and from September to the end of December	10-15 sheep ha^{-1} in the months of April, September and October; intermittent grazing between January and March	5 sheep ha^{-1} all year round
Supplementary feeding	None	None	hay	none
Vegetation cutting date	Cut in late July or early August (e.g. 23rd July 2016) for hay	Cut in July (e.g. 23rd July 2016) for silage	Cut in mid to late July or early August (e.g. second week of August 2016) for hay	N/A

FYM = farmyard manure, Permanent = permanent grazing, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, N/A = not applicable.

3.2.2 Experimental design, soil sampling and analysis

As multiple fields with the two contrasting management regimes and soil types could not be found at each location, we established five 25 m^2 replicate plots within single fields. The plots were part of an ongoing study to monitor gaseous land-atmosphere C fluxes. Soil samples were collected in May and June 2016. Within each plot, soil samples (0 – 15 cm depth) were randomly collected from five points with a soil auger (5 cm diameter) and bulked into a composite sample, giving five replicate samples per site at each location. One additional augered sample was collected from each 25 m^2 plot to determine bulk density of the 15 cm core depth (N = 5 per management). Samples were transported and stored at 4°C prior to analysis at the School of Geography, University of Leeds, UK.

Soil samples were analyzed using standard laboratory techniques. A small proportion of each of the fresh soil samples was used for the determination of moisture content and available nitrogen (ammonium – nitrogen, NH_4-N; and nitrate – nitrogen, NO_3-N) within three days of sample collection. Moisture content was determined by measuring the weight loss after drying the fresh soil samples in an oven at 105°C for 12 hours. Available N was extracted from the fresh soil samples with 1M potassium chloride (KCl) and analyzed within 24 hours using a Skalar SAN++ auto analyzer. Bulk density was determined by drying core samples in an oven overnight and dividing the weight of dry soil by the volume of the core occupied by the soil after correction for stoniness (percentage of rock fragments greater than 2 mm in diameter; Poeplau *et al.*, 2017).

$$Bulk\ density\ (g\ cm^{-3}) = \frac{Mass\ of\ dry\ soil - Mass\ of\ stones}{Volume\ of\ soil - \left(\frac{Mass\ of\ stones}{Density\ of\ stones\ (2.6\ g\ cm^{-3})}\right)} \qquad 3.1$$

The remaining soil samples were air dried and passed through a 2 mm sieve, after visible roots and other plant materials were removed. The sieved samples (< 2 mm) were used for the determination of all other soil physico-chemical properties. Soil pH was measured in a soil suspension of 1:2.5 soil-water ratio (Robertson *et al.*, 1999). Total P was determined using a Skalar San ++ continuous flow auto analyzer, after the soil samples were digested using sulphuric acid and hydrogen peroxide (Tiessen and Moir, 1993). Total C and N were measured by combustion in an elemental analyzer (Vario Micro Cube), after the soil samples were first ball milled and passed through a 0.50 mm sieve. The total C values were used as SOC because the soil samples were free from carbonates when tested with hydrochloric acid (HCl). Exchangeable basic cations (calcium, Ca; magnesium, Mg; potassium, K; and sodium, Na) and aluminium (Al) were extracted with 1 M ammonium acetate (Allen, 1989) and analyzed using a Thermo Scientific iCAP 7600 Duo ICP-OES Analyzer. For particle size analysis, soil samples were dispersed using 5% sodium hexametaphosphate and passed through 0.53 mm sieve to separate the sand particles; the clay fraction was then determined by pipette method and the silt fraction calculated by subtraction (Van Reeuwijk, 2002). SOC stocks (Mg ha^{-1}) were calculated from values of SOC concentration and bulk density using the relation:

$$SOC\ stock\ (Mg\ ha^{-1}) = C\ \times BD\ \times D\ \times (1 - stone\ fraction) \qquad 3.2$$

Where C = SOC concentration (%), BD = bulk density (g cm^{-3}), D = soil depth (cm). As the soil bulk density of the four sites differed, we performed a soil mass correction by calculating the mass of soil within 0 – 15 cm depth of the site with the highest bulk density and calculating the stock of SOC in the equivalent soil mass in the other sites (Lee *et al.,* 2009).

3.2.2.1 SOC fractionation

SOC fractionation was carried out following the method of Zimmermann *et al.* (2007) and the standardization of some of its stages by Poeplau *et al.* (2013). A calibrated ultrasonic probe with an output energy of 22 J ml^{-1} was used to disperse 30 g of soil sample (< 2 mm) in 150 ml of deionized water. The dispersion time was 177 s in order to maintain an effective fixed amount of power (20 W) (Poeplau *et al.,* 2013). The dispersed suspension was transferred into a 63 µm aperture sieve bags clamped to a wet sieve shaker (this made the sieving process easier and quicker). The suspension was wet sieved until the rinsing water was clear. In order to avoid the effect of the amount of sieving water on DOC, a minimum of 2000 ml of water was used for wet sieving (Poeplau *et al.,* 2013). The sand and stable aggregates (S+A) (> 63 µm) together with particulate organic matter (POM) retained on the sieve, was dried at 40°C and weighed. The suspension (< 63 µm) was filtered through a 0.45 µm aperture nylon sieve and the material greater than 0.45 µm, silt and clay (s+c), was dried at 40°C and weighed. Filtration through the 0.45 µm aperture nylon sieve was accomplished using centrifuge. The 0.45 µm sieve was placed over a 1200 ml centrifuge containers and slightly pushed into the container to create a depression where the suspension (< 63 µm) was poured. The container was covered and then centrifuged at 1000 g for 5 minutes. This gave a very clear separation. An aliquot of the filtrate (< 0.45 µm) was analyzed for DOC by thermal oxidation with a liquid analyzer (Analytik Jena Multi N/C 2100).

The S+A was stirred in sodium polytungstate at a density of 1.8 g cm^{-3} in order to separate POM. The mixture was centrifuged at 1000 g for 15 minutes and the light fraction (POM) decanted. The two fractions (S+A and POM) were washed with deionized water to remove all traces of sodium polytungstate, dried at 40°C and weighed.

A chemically resistant soil organic carbon (rSOC) fraction was extracted from the s+c fraction by sodium hypochlorate (NaOCl) oxidation. One gram (1 g) of s+c fraction was oxidized for 18 hours at 25°C in a water bath with 50 ml of 6% NaOCl adjusted to pH 8 with HCl. The oxidation residue was centrifuged at 1000 g for 15 minutes, decanted, washed with deionized water and centrifuged further. The oxidation process was repeated twice.

After the fractionation, the C and N contents of S+A, s+c, POM and rSOC, were measured by combustion with an elemental analyzer (Vario Micro Cube). The C fractions were further grouped into three classes: the labile pool (POM and DOC), the physically protected pool (S+A and s+c – rSOC) and chemically resistant pool (rSOC) (Figure 3.1). In order to assess the relative sizes of C fractions, the C content in the measured mass of each fractional pool was calculated. This enabled us to determine the percentage recovery of C. The OC contained in the different fractional pools were then expressed as a percentage of the total SOC of the bulk soil. This revealed what proportion of the grasslands' total SOC was distributed in the different fractional pools.

3.2.3 Statistical analysis

The distributions of all the data to be analyzed were assessed using Shapiro-Wilk normality test. As all the data were normally distributed ($p > 0.05$), they were subjected to parametric tests. One-way analysis of variance (ANOVA) was used to compare the means of soil parameters between the four sites. Homogeneity of variance was assured by Levene's Test and post hoc multiple comparison (Tukey HSD) was used to separate means of soil parameters that differed significantly between fields.

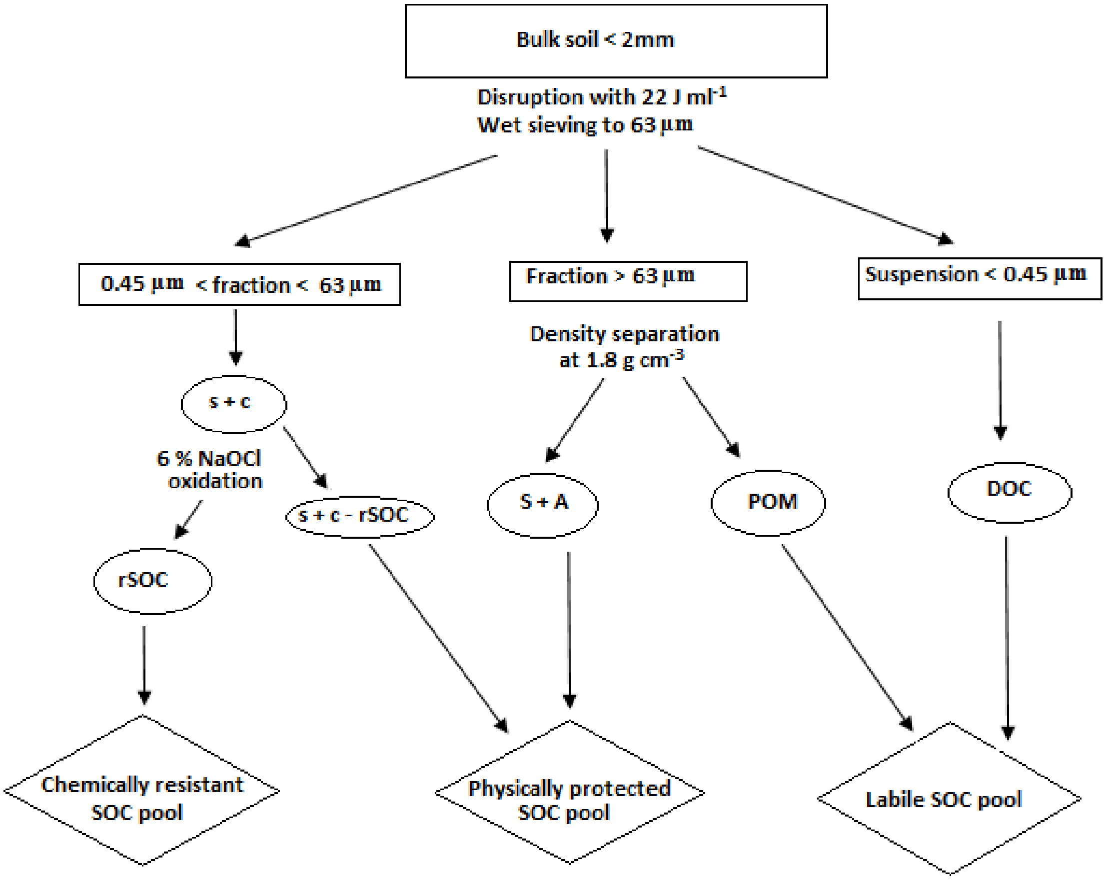

Figure 3.1: Flow chart showing SOC fractionation stages and their products (modified from Zimmermann *et al.*, 2007); DOC = dissolved organic carbon, POM = particulate organic matter, S+A = sand and stable aggregates, and s+c = silt and clay.

3.3 Results

Results of selected soil physical and chemical properties including SOC and its fractions are presented in Tables 2 to 4. There was no significant difference ($p > 0.05$) in some soil attributes such as clay content, exchangeable aluminium (Al^{3+}), exchangeable calcium (Ca^{2+}) and exchangeable magnesium (Mg^{2+}) between the two locations (Table 3.2). Nidderdale soils contained higher exchangeable potassium (K^+), nitrate – nitrogen (NO_3-N) and sand with the textural class being sandy loam, and had lower silt content, stoniness and ammonium nitrogen (NH_4-N) compared to the Ribblesdale soils which were characterized by a loam texture. In Ribblesdale, there was no significant difference in pH, exchangeable sodium (Na^+), total phosphorus (P), total nitrogen and NH_4-N between the soils under hay meadow and permanent pasture. On the other hand, the hay meadow soil in Nidderdale had significantly higher pH and significantly lower exchangeable Na^+, total P and total N than the silage pasture soil in Nidderdale and the two fields in Ribblesdale (Table 3.2). The hay meadow soils in the two locations had higher bulk density compared to the conventional pasture soils, however, this was only statistically significant at Nidderdale.

The SOC stock in all the sites investigated ranged from 58.93±3.50 to 100.69±8.6 Mg ha^{-1}, and was significantly higher ($p < 0.05$) in the Nidderdale silage pasture (100.69±8.6 Mg ha^{-1}) than in the Nidderdale hay meadow (58.93±3.50 Mg ha^{-1}) and both sites at Ribblesdale (64.58±6.29 to 68.74±3.15 Mg ha^{-1}) (Table 3.3). The Ribblesdale sites had similar C/N values which were significantly lower than those in the Nidderdale sites, and the soils of the silage pasture in Nidderdale had the highest C/N (14.62±0.21).

The percentage recovery after fractionation of the soil into five pools (S+A, s+c – rSOC, rSOC, POM and DOC) were 91 – 98% of the total soil mass and 61 – 81% of total SOC (Tables 3 and 4). The average contribution of the different soil fractions to the recovered soil mass was as follows: S+A fraction (78.62%), s+c fraction (16.62%) and POM fraction (0.20%) whereas the average concentration of C in the different fractions was in the decreasing order of POM (38.74%) > s+c – rSOC (4.24%) > S+A (3.38%) > rSOC (2.39%) > DOC (0.03%) (Table 3.4). The percentage of SOC stock that resided in the S+A fraction was the highest (an average of 48.0%) of all the fractions and at all the four sites studied; the percentage of the SOC stock in the other fractions were in the decreasing order: s+c – rSOC

(12.0%) > rSOC (7.0%) > POM (1.4%) > DOC (0.4%) (Table 3.3). The C/N values were highest in the POM fraction and decreased in the order of s+c – rSOC > S+A > rSOC. Across all the locations and management regimes studied, there was no significant difference in the percentage of SOC stock residing in s+c – rSOC, rSOC and POM. The Ribblesdale permanent pasture had the highest percentage of SOC stock (62.40%) in the S+A fraction, and the Nidderdale silage pasture had the lowest SOC stock in this fraction (38.08%). The percentage of SOC stock in DOC fraction was generally higher in Nidderdale than in Ribblesdale, but only in Nidderdale hay meadow was the difference statistically significant.

The results of grouping the SOC pools into three classes, based on their relative stability in the soil and the mechanism of storage are shown in Figure 3.2. Across all the sites studied, the distribution of SOC stock was an average of 60.31% in physically protected pool, 6.98% in chemically protected pool and 1.77% in labile pool. About 30.94% of the bulk soil OC stock was not recovered during the fractionation process. The proportion of SOC stock in chemically protected and labile pools did not differ significantly between the sites studied. On the other hand, a significantly higher percentage of SOC stock (73.12%) was physically protected in the permanent pasture in Ribblesdale than in the silage pasture in Nidderdale (52.49%). There was no difference in physically protected SOC between hay meadows.

Table 3.2: Mean values ± standard error (n=5) of selected soil physico-chemical properties.

Soil property	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
Sand (%)	65.20±1.69a	60.60±1.50a	50.00±0.84b	49.60±1.40b
Silt (%)	24.20±4.51a	29.60±3.12ab	43.00±0.45b	36.60±4.63ab
Clay (%)	10.60±3.78a	9.80±2.75a	7.00±1.22a	13.80±5.38a
Textural class	Sandy loam	Sandy loam	Loam	Loam
Stoniness (%)	0.77±0.13a	1.02±0.18a	0.96±0.34a	3.9±1.00b
Bulk density (g cm^{-3})	0.84±0.03a	0.63±0.03b	0.72±0.03ab	0.66±0.05b
pH in water	6.06±0.04a	5.72±0.12b	5.64±0.03b	5.81±0.10ab
Ex-Al (mmol kg^{-1})	0.04±0.01a	0.10±0.06a	0.13±0.03a	0.07±0.03a
Ex-Ca (mmol kg^{-1})	41.44±0.89a	41.62±1.80a	36.01±0.47a	37.34±2.42a
Ex-Mg (mmol kg^{-1})	11.16±0.47a	10.03±0.42a	9.41±0.65a	11.89±1.04a
Ex-K (mmol kg^{-1})	1.95±0.19a	3.44±0.38b	0.74±0.09c	0.84±0.24c
Ex-Na (mmol kg^{-1})	0.72±0.04a	1.45±0.21b	1.22±0.06b	1.11±0.05ab
Total P (mg kg^{-1})	928.69±66.64a	1533.63±107.86b	1345.67±46.77b	1438.50±74.48b
Total N (%)	0.37±0.02a	0.54±0.03b	0.55±0.03b	0.55±0.04b
NH_4-N (mg kg^{-1})	1.49±0.45ab	0.69±0.09a	2.44±0.32b	1.61±0.21ab
NO_3-N (mg kg^{-1})	1.160±0.32a	1.23±0.10a	0.23±0.06b	0.71±0.12ab

Ex = exchangeable, Al = aluminium, Ca = calcium, Mg = magnesium, K = potassium, Na = Sodium, P = phosphorus, N = nitrogen, NH_4 = ammonium, NO_3 = nitrate. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

Table 3.3: SOC and C/N in total soil mass and in soil fractions (mean values ± standard error, n=5).

Soil fraction	Property	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
Total soil mass	Total SOC (%)	4.70±0.22a	7.99±0.49b	5.53±0.24a	5.37±0.35a
	SOC stock (Mg/ha)	58.93±3.50a	100.69±8.64b	68.74±3.15a	64.58±6.29a
	C/N	12.66±0.16a	14.62±0.21b	10.04±0.81c	9.95±0.22c
S+A	% of total SOC	43.55±8.11ab	38.08±5.26a	47.83±2.79ab	62.40±2.58b
	C/N	12.33±0.50a	14.11±0.32b	9.60±0.09c	9.57±0.16c
s+c – rSOC	% of total SOC	13.65±3.99a	14.41±4.83a	10.60±1.49a	10.72±0.57a
	C/N	9.10±1.42a	11.24±1.29a	8.23±0.27a	8.08±0.28a
rSOC	% of total SOC	8.94±1.67a	7.14±1.30a	4.72±0.68a	7.10±1.36a
	C/N	18.60±1.54a	23.02±2.02a	12.80±0.60b	12.83±0.57b
POM OC	% of total SOC	1.56±0.18a	1.31±0.25a	1.28±0.20a	1.35±0.13a
	C/N	23.12±4.45a	22.04±0.92a	24.13±0.39a	22.58±1.82a
DOC	% of total SOC	0.60±0.03a	0.44±0.06ab	0.24±0.03b	0.30±0.07b
Total SOC recovered (%)		68.30±8.75a	61.30±3.65a	64.66±3.38a	81.86±2.93a

SOC = soil organic carbon, N = nitrogen, S+A = sand and stable aggregate fraction, rSOC = resistant SOC fraction, s+c – rSOC = silt and clay without the rSOC fraction, POM = particulate organic matter fraction, DOC = dissolved organic fraction. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

Table 3.4: The organic C contents and soil masses of different soil fractions (mean values ± standard error, n=5).

Soil fraction	Property	Nidderdale-Hay	Nidderdale-Silage	Ribblesdale-Hay	Ribblesdale-Permanent
S+A	OC content (%)	2.33±0.34a	3.72±0.66ab	3.21±0.14ab	4.24±0.29b
s+c – rSOC	OC content (%)	4.38±1.18a	6.56±1.54a	3.25±0.39a	2.78±0.18a
rSOC	OC content (%)	2.90±0.38ab	3.41±0.40a	1.44±0.18c	1.79±0.29bc
POM OC	OC content (%)	37.38±5.10a	42.10±0.46a	38.36±1.74a	37.12±2.20a
DOC	OC content (%)	0.03±0.001a	0.04±0.005a	0.01±0.001b	0.02±0.003b
S+A	% of total soil mass	83.64±1.15a	80.47±2.51ab	75.57±1.13b	74.79±0.90b
s+c	% of total soil mass	13.92±1.33a	16.27±2.20a	16.47±0.36a	19.80±1.64a
POM	% of total soil mass	0.21±0.03a	0.24±0.04a	0.17±0.02a	0.19±0.02a
Total soil mass recovered (%)		97.77±0.33a	96.97±0.96a	92.23±1.26b	94.77±1.00ab

SOC = soil organic carbon, S+A = sand and stable aggregate fraction, rSOC = resistant SOC fraction, s+c – rSOC = silt and clay without the rSOC fraction, POM = particulate organic matter fraction, DOC = dissolved organic fraction. Row means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

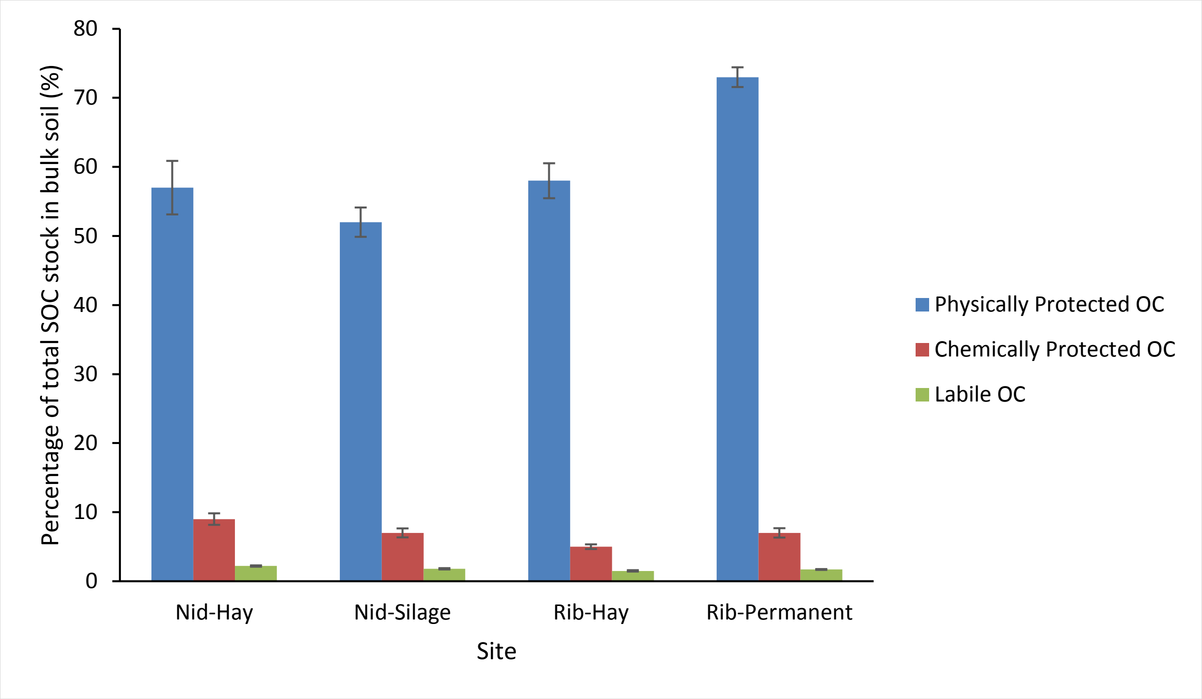

Figure 3.2: Distribution of SOC stock in three fractional classes: physically protected OC pool (S+A and s+c – rSOC), chemically protected OC pool (rSOC), and labile OC pool (POM and DOC), as a percentage of total SOC stock in bulk soil. About 30% of the total OC stock in bulk soil was not recovered during fractionation. Bars represent mean values ± standard errors. Nid = Nidderdale and Rib = Ribblesdale.

3.4 Discussion

The range in organic C stock of soils (0-15 cm) reported here (58.93±3.50 to 100.69±8.6 Mg ha^{-1}) is similar to the range previously reported for grasslands in Britain. For example, Bradley *et al.* (2005) reported a mean surface (0-30 cm depth) SOC stock of 80 Mg ha^{-1} for pasture lands in England, and Carey *et al.* (2008) reported an average of 69 (61 to 82) Mg ha^{-1} C stock for British grassland soils (0 – 15 cm) in the Countryside Survey of 2007. Similarly, Ward *et al.* (2016) reported mean surface (0-20 cm) SOC stocks of 82.6 Mg ha^{-1} and 84.7 Mg ha^{-1} for 60 intensively managed and 60 extensively managed grasslands in England. This indicates that the upland grasslands in both Nidderdale and Ribblesdale are important C stores with implication for delivering various ecosystem services including climate change mitigation.

3.4.1 Effects of grassland management and soil parent material on soil organic C stocks

The Nidderdale site under silage pasture had a significantly higher SOC stock (100.69±8.64 Mg ha^{-1}) compared to other sites. This did not support our first hypothesis which expected a greater C stock in hay meadow fields under agri-environmental schemes with intermittent grazing. However, at our field sites, the differences in stocking rates that are likely to affect vegetation removal and input of organic materials to the soil, and which ultimately determine the C balance of the ecosystem, were relatively small. The key management difference between the sites that could explain the greater SOC stock in the Nidderdale silage pasture was the addition of inorganic N fertilizers (50 kg N/ha/year). Firstly, increased forage productivity due to fertilizer application might have led to a greater return of plant litter to the soil of the Nidderdale silage pasture, despite offtake via cutting for silage, compared to the other sites with lower or no fertilizer inputs. It is also likely that there was a greater belowground biomass at the Nidderdale silage pasture due to N-induced increase in the network of roots. A number of studies have shown that added N stimulates vegetation growth and the return of biomass litter to the soil, and subsequently enhances soil C storage (Amman *et al.,* 2009; Fornara *et al.,* 2013; Liang and Balser, 2012; Song *et al.,* 2014; Yue *et al.,* 2016). In addition, the significantly higher C/N (14.62±0.21) in the Nidderdale silage pasture may limit the rate of decomposition of organic matter, thereby resulting in the accumulation of C. This was shown by Hassink (1994) who found a negative correlation between the rate of organic matter mineralization and C/N values ranging from 10 to 18 in grassland soils. A high C/N is generally associated with low quality of organic matter which renders it more resistant to microbial decomposition in the soil (Berg and Laskowski, 2006; Yue *et al.,* 2016).

Contrary to our second hypothesis, there was no significant difference in SOC stocks between the Nidderdale soils (59 - 101 Mg ha^{-1}) with base-poor parent material and the Ribblesdale soils (65 - 69 Mg ha^{-1}) with limestone parent material, although the range was greater at Nidderdale possibly due to N fertilizer addition. The lack of difference between locations could be attributed to similar soil pH levels, most likely as a consequence of liming of the Nidderdale sites. Although the hay meadow field in Nidderdale had a significantly higher pH compared to all the other sites ($p < 0.05$), the soils at all sites were moderately acidic with pH values ranging from 5.64 to 6.06; this is similar to an average pH of 6.06 reported for improved grasslands in Britain in 1998 (Carey *et al.,* 2008). At this pH range, the soil's exchange complex is mostly saturated with Ca^{2+} and Mg^{2+} while Al^{3+} concentration is reduced (Paradelo *et al.*, 2015). This is consistent with the concentrations of exchangeable polyvalent cations observed in our two locations: high Ca^{2+} and Mg^{2+}, and low Al^{3+}. The concentration of Al^{3+} in the soil is often associated with soil C stability. For example, Haynes and Naidu (1998) explained that at a lower soil pH than observed in our sites, and with higher Al^{3+} concentration, acid soils are normally flocculated because of the attraction between Al^{3+} and negatively charged clay surfaces, as well as the adsorption of OM. Liming raises pH, causing the Al^{3+} ions to precipitate and leaving a net negative charge on the soil surfaces. The resultant dominance of repulsive forces disperses the soil particles, thereby releasing adsorbed OM and exposing it to microbial decomposition. This was perhaps the case in the limed Nidderdale sites, resulting in the reduction of soil C to the levels observed at the Ribblesdale sites. The result obtained in this study therefore indicates that soil management activities such as liming has the capacity to change any effects of parent material on soil chemistry and thus C dynamics. This needs to be considered in soil management and climate mitigation strategies so as to minimize soil C loss.

Our study has shown that although grasslands may be subjected to different management regimes such as traditional hay meadow under agri-environment scheme and conventional permanent pasture and silage pasture, in practical terms the stocking rates, vegetation removal and indirect fertilization via animal droppings may be similar and application of inorganic and organic fertilizers may have comparable effects, thereby causing similar effects on the soil ecosystem. The continuous low intensity grazing at the Ribblesdale permanent pasture possibly offsets the lack of organic manure inputs, thereby explaining why there was no significant difference in SOC stock between the hay meadow and the permanent pasture field at this location. It is therefore necessary for future grassland management policies to specify the exact combination of management activities that define a management regime if soil C accrual is one of the target benefits. This will ensure that the efficacy of agri-environment policy for multiple environmental benefits can be adequately assessed across sites and geographical regions.

3.4.2 Effects of grassland management and soil parent material on soil C fractions

Consistent with our third hypothesis, a greater proportion (67.29%) of SOC stock was protected in relatively stable forms, with physically protected pools associated with soil mineral fractions accounting for 60.31%. This is somewhat lower than has been found for mineral soils elsewhere. For instance, Leifeld and Fuhrer (2009) studied C distribution within the surface (0-20 cm) of some clay loamy soils under pasture and hay meadow management in Switzerland; they found only less than 20% of the SOC in the POM fraction whereas over 80% of the C was associated with the mineral fractions. This is despite slightly higher SOC contents (10%) compared to soils studied here (4.7 – 8.0% C). Similarly, in a silty loam (73% silt and 16% clay) grassland in Germany with high fertilizer inputs (160 kg N ha^{-1} $year^{-1}$) and cut four times a year, John *et al.* (2005) found that 86-91% of SOC was associated with the mineral soil fraction.

The distribution of SOC stock in all other soil fractions, except sand and stable aggregate fraction (S+A), was similar across the four sites we studied. The SOC stock in S+A was similar between the two management regimes in each location, and in the hay meadows of the two locations but was greater in the permanent pasture of Ribblesdale than in the silage pasture of Nidderdale. This indicates an effect of soil aggregate stability rather than management regime or soil parent material. The permanent pasture in Ribblesdale with a higher C stock in its S+A fraction had lower sand and higher clay content compared to other sites. Intuitively, one would expect a lower C stock in the S+A fraction of the Ribblesdale permanent pasture due to lower sand content, however, the stable aggregate component of the fraction which is a function of finer particles such as clay with greater surface area, is known to have a high capacity for C occlusion (Hassink and Whitmore, 1997). The relatively higher clay contents in the Ribblesdale permanent pasture might have induced a greater soil aggregate stability thereby leading to a higher SOC stock to be occluded in their S+A fraction. This is corroborated in a number of studies where soil aggregate stability was shown to increase with increasing clay contents and decreasing sand contents (Aziz and Karim, 2016; Lado *et al.*, 2004), resulting in an increase in SOC stock (Le Bissonnais and Arrouays, 1997; Arrouays *et al.*, 2006). Our study therefore highlights the need to always consider the particle size distribution of soils irrespective of their parent materials and site management activities when fractional distribution of soil C is being studied.

3.4.3 The significance of soil C distribution to environmental change

In all the sites studied, about 60% of SOC stock is stored in physically protected pools. This indicates that the mineral soils of both Nidderdale and Ribblesdale grasslands have large stocks of stable SOC with potentially long mean residence times important for climate change mitigation. For example, Leifeld and Fuhrer (2009) reported mean residence times of 210 to 795 years for physically protected C pools compared to 5.5 to 11.5 years for labile soil C pools in a Swiss grassland under hay meadow and pasture management regimes. The apparent beneficial effects to climate change mitigation of the grasslands we studied may however be negated depending on the fate of the labile C pool. Although only about 2% of the SOC stock in our study is present in labile pools, it is likely that the proportion of SOC stock that were not recovered after fractionation (19 to 39%) was mostly labile. We suspect that labile C, particularly the POM fraction was lost during the wet sieving and decantation stages of the fractionation process, at the following steps: 1) during wet sieving, suspended POM could have escaped from the sieve bags due to agitation caused by the mechanical shaker; 2) when the sodium polytungstate suspension was being decanted from the S+A fraction, and 3) when sodium polytungstate was being washed off the POM and S+A fractions. An additional likely source of C loss is the recovery of s+c fraction after filtering the suspension (< 63 µm) through a 0.45 µm aperture nylon sieve. This is possible as very fine clay particles containing OC could be retained on the sieve. Despite its lower fractional mass (0.2% of soil mass compared to 94.8% of mineral soil mass recovered), the labile C fraction particularly POM had a significantly higher OC concentration (39%) than the mineral fraction (7%). It is therefore possible that if the labile fraction accounts for the 5% loss in soil mass, it could contain about 20% of SOC stock lost during fractionation. The dynamics of this labile C will be critical to the climate system when regional and global scales are considered.

The general view that the labile C pool is highly vulnerable to environmental change is due to its short mean residence time (Leifeld and Fuhrer, 2009), hence it is considered to be affected by management activities such as grazing and fertilizer application (Cao *et al.*, 2013;

Riggs *et al.,* 2015). In our study, despite the inorganic fertilizer-induced higher SOC stock in the Nidderdale silage pasture, there was no significant difference in the distribution of the labile C fractions between the Nidderdale silage pasture and the other three fields. This could mean that the benefits of the higher SOC stock in the Nidderdale silage pasture may be short-term. In a wider study of UK grasslands, Ward *et al.* (2016) found that labile SOC in the surface soil (to 7.5 cm depth) decreased with increasing management intensity, whereas the stable pools remained unaffected. In the Ward *et al.* (2016) study no differentiation was made between upland and lowland grassland sites, and it is possible that the management effect on labile C is less pronounced in upland environments such as in this study where climatic conditions are less favourable for productivity. This has implications for a changing climate, and may mean that rapid C turnover in upland sites is relatively robust to subtle changes in management.

There is therefore a need to further investigate the fate of the labile C pool in our sites because it is the most active soil C pool. A number of questions that need to be answered are: 1) What are the turnover rates of C in the labile pools? 2) What are the products of turnover of the labile C pools? Providing answers to these questions is important because similar distributions and sizes of the labile C pools in our sites do not necessarily imply the same dynamics. Similar pool sizes may be due to different C flux patterns such as high input – high output of C or low input – low output of C in the system. The pattern of C flux and the nature of the output such as CO_2, CH_4 or DOC are particularly important as they could affect climate feedbacks, and have other environmental effects. For example, under high input – high output scenario where the major output is CO_2, the radiative effects of high efflux of CO_2 into the atmosphere may be balanced by the positive physiological and fertilization effects on vegetation, which enhances soil C accumulation (Bala *et al.,* 2013). On the other hand, if the major output is CH_4 under the same high input – high output scenario, there is a greater risk of an increased radiative effects. This is because the capacity of CH_4 to absorb infrared radiation as a greenhouse gas is 20 to 30 times more efficient than CO_2 (Mer and Roger, 2001). High DOC outputs from the upland grasslands may be costly for water treatment companies if the DOC ends up in river networks that are drinking water sources, and have consequences for aquatic ecosystems (Chapman *et al.,* 2010). Conducting C flux studies in the upland grasslands investigated in this study will therefore help to improve understanding of the dynamics of C in their soils as well as potential implications for climate change.

3.5 Conclusion

The upland grasslands in both Nidderdale and Ribblesdale stored a large amount of C in their soils which ranged from 58.93±3.50 to 100.69±8.64 Mg ha^{-1}. There was little contrast between sites except that the only field that received inorganic N fertilizer (Nidderdale silage pasture) had a significantly higher SOC stock than the other three fields studied. The activities that constitute grassland management regimes need to be considered carefully in agri-environment policies in order to be able to assess their net benefits to the ecosystem. No effects of parent material was observed on soil C stock due to liming-induced increase in the pH of Nidderdale soils, and neither management activities nor parent materials affected the distribution of soil C into different fractions. About 70% of the SOC stock was protected within the stable soil fractions in all the sites studied, indicating a potential for the grasslands to contribute in climate change mitigation. However, there is need to further study the dynamics of the labile SOC fraction which has short residence time and could negate the beneficial effects of the protected C pools in a changing climate. Further studies should focus particularly on the turnover rates of the labile C pools as well as the outputs from the turnover such as CO_2, CH_4 and DOC. This is expected to help in better understanding of not just the dynamics of the labile soil C pools but also the implication of such dynamics for climate change.

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Chapter 4: Upland grasslands in northern England were atmospheric carbon sinks regardless of management regimes

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Abstract

Continuous exchange of C in the forms of CO_2 and CH_4 occurs between the atmosphere and the terrestrial ecosystem. These GHGs contribute significantly to global warming when present in the atmosphere. Thus, there is growing interest in understanding better ways of managing terrestrial ecosystems so as to increase or fully utilize their capacity to sequester GHGs, or reduce emissions, and mitigate climate change. In this study, we examined the fluxes of CO_2 and CH_4 in two upland grassland locations (Nidderdale and Ribblesdale) in northern England with contrasting lithologies and under: 1) traditional hay meadow and 2) conventional pasture management. NEE and ER were measured for 12 months from June 2016 to May 2017, alongside other environmental variables such as soil temperature and moisture, and photosynthetically active radiation (PAR). Results showed that the grasslands were a net atmospheric C sink, with an uptake of 1822 – 2758 g CO_2-eq m^{-2} $year^{-1}$. This C uptake is greater than those reported in other European grasslands due to low ER. The Ribblesdale hay meadow had the lowest C uptake (1822 g CO_2-eq m^{-2} $year^{-1}$) likely due to low available soil N resulting from the absence of N fertilization. This has implication for agri-environment schemes that discourage the use of inorganic N fertilizers. Warmer condition in Ribblesdale was implicated as the cause of higher C efflux relative to

Nidderdale, which has implications for future climate change. The CH_4 fluxes were very low (-0.36 to -0.44 g CH_4 m^{-2} $year^{-1}$) and did not differ significantly between management regimes. It is recommended that future research should prioritise the overall GHG balance of upland grasslands and their inter-annual and decadal variability.

Keywords: Carbon flux; atmospheric carbon; carbon sequestration; grassland management; upland soils; climate change

4.1 Introduction

Removing and storing atmospheric C by the terrestrial ecosystem is a key strategy that has been recognised widely for mitigating climate change. The terrestrial ecosystem takes up atmospheric CO_2 via photosynthesis and releases it back into the atmosphere through respiration. CH_4 is also taken up by aerobic soils (Lowe, 2006) and released into the atmosphere under anaerobic conditions (Van den Pol-van Dasselaar *et al.,* 1999). The net balance between these processes of C uptake and C release determines an ecosystem's potential contribution to climate change. NEE, the difference between CO_2 uptake via photosynthesis (GPP) and CO_2 released by respiration (ER), is an important measure of C flux that has been used to assess the C sink-source status of terrestrial ecosystems (Chen *et al.,* 2013; Kang *et al.*, 2013; Moinet *et al.*, 2016). Net CH_4 flux (expressed as CO_2 equivalent – CO_2-eq) can be added to NEE to give a better understanding of C sink-source status and global warming potential (GWP).

The NEE of grasslands varies greatly, with both negative (C sink) and positive (C source) values reported in different parts of the globe (Novick *et al.,* 2004). In Europe, for example, annual NEE of grasslands ranges from a net source of +627 g CO_2 m^{-2} to a net sink of -2394 g CO_2 m^{-2} (Table A4.1). Although annual and decadal variability in grasslands' NEE occurs, under similar management and environmental conditions, this variability is usually small (Jacobs *et al.,* 2007; Peichl *et al.,* 2011). The large variability in NEE between grasslands has therefore been attributed to the effects of management activities and environmental characteristics such as precipitation, soil type, soil temperature and moisture (Jacobs *et al.,* 2007; Polley *et al.,* 2008). However, the direction of reported management effects on C flux is not consistent because grasslands subjected to specific management activities such as grazing (Haferkamp and MacNeil, 2004; Kjelgaard *et al.,* 2008; Owensby *et al.*, 2006;

Skinner, 2008) and fertilization (Bardgett and Wardle, 2003; Fang *et al.,* 2012; Harpole *et al.,* 2007; Welker *et al*., 2004; Xia *et al.,* 2009) still have both negative and positive NEE values. Under similar environmental conditions and management duration, differences in management effects are most likely due to management intensity which differs across grasslands and influences a range of ecosystem processes that determine the net C flux. For example, grazing intensity and livestock stocking density influence the net C flux in grasslands (Klumpp *et al.,* 2007) through effects on aboveground biomass offtake, residue accumulation, manure distribution, soil compaction and aeration, and soil temperature and moisture, all of which create optimal or unfavourable conditions for plant growth and soil microbial activities (Chiavegato *et al.,* 2015). The rate of fertilizer application to grasslands similarly influences net C flux by stimulating plant growth and microbial decomposition (Ammann *et al.,* 2007; Schmitt *et al.,* 2010; Skiba *et al.,* 2013; Zeeman *et al.,* 2010).

Determining the exact effect of management activities on C flux is further complicated because managing grasslands often involves a combination of activities such as inorganic fertilizer application, manure addition, cutting of vegetation for hay or silage and/or direct grazing by livestock (e.g. Table A4.1). Additionally, these management activities interact with environmental factors. For example, N addition stimulates plant productivity e.g. increase in gross ecosystem photosynthesis (Xia *et al.,* 2009), leaf area index and shoot/root ratio (Cheng *et al.,* 2009) but this is only effective when there is ample supply of water to the ecosystem (Harpole *et al.,* 2007). The C sequestration potential of grasslands is also influenced by the nature of their underlying soils and the material from which the soils were formed (soil parent material). Managed grasslands with mineral soils tend to sequester more C than managed grasslands on organic soils (Jacobs *et al.,* 2007). This happens because mineral soils offer physical protection to organic C via encapsulation within aggregates and adsorption by clay minerals (Jones and Donelly, 2004). Physical protection of organic C is lacking in organic soils, rendering them more susceptible to microbial decomposition of OM and C release, depending on soil moisture conditions. Soil parent material is considered an important factor in C flux because it influences soil physical and chemical properties (Shrestha *et al.,* 2014). For example, acid-forming parent materials such as siliceous stones and alkaline-forming parent materials such as limestones exert significant control on the pH of their resulting soils (Mijangos *et al.,* 2010), and soil pH correlates positively with soil

CO_2 efflux (Chen *et al.,* 2016). Soil parent material also exerts a strong influence on soil texture and mineralogy (Araujo *et al.,* 2017), which determine the surface area of soil particles available for organic C occlusion as well as soil moisture retention and availability to both plants and soil microbes.

Despite the complex interactions between management and environmental conditions, there is an emerging pattern of C flux in managed grasslands. Net uptake of atmospheric C (i.e. negative NEE values) has been reported mostly in managed grasslands underlain by mineral soils (Jacobs *et al.,* 2007) and where soil moisture was not limiting for plant growth (Hao *et al.,* 2013; Rigge *et al.,* 2013). On the other hand, net C efflux (i.e. positive NEE) in managed grasslands has been associated with organic soils (Gilmanov *et al.,* 2007), seasonal increase in soil temperature (Cui *et al.,* 2014; Frank *et al.,* 2002; Jones *et al.,* 2006), an increase in soil pH (Chen *et al.,* 2016), and when soil moisture available for plant growth is reduced during periods of high evapotranspiration and low precipitation (Novick *et al.,* 2004; Wang *et al.,* 2016). However, it is not yet clear how different management regimes affect C flux in grasslands that are characterized by mineral or organo-mineral soils formed from different parent materials but under similar environmental conditions such as temperature and moisture.

Over time, and under constant management and environmental conditions, the C sequestration potential of grasslands will reach an equilibrium level or saturation point (Six *et al.,* 2002; Smith, 2014). However, until the saturation point for a grassland is attained, appropriate management practices are needed to ensure that the C sequestration potential is fully achieved. Even after saturation, management practices that prevent the loss of accumulated C will still be necessary. There is still significant potential to increase C sequestration in temperate grasslands (Jones and Donelly, 2004) and poorly managed grasslands can be improved to increase its C sink capacity and also protect its C stock from loss (Smith, 2014). By identifying and adopting management practices that enhance C sequestration, grasslands will contribute significantly in climate change mitigation (Rogiers *et al.,* 2005). Intensively managed grasslands reportedly had a greater C uptake (NEE = -848 g CO_2 m^{-2} $year^{-1}$) compared to extensively managed grasslands (NEE = -239 g CO_2 m^{-2} $year^{-1}$) during 1997 – 2006 period in some parts of Asia, Europe and North America (Gilmanov *et al.,* 2010). This was most likely because the intensively managed grasslands were more productive with an average GPP of 5767 g CO_2 m^{-2} $year^{-1}$ than the extensively managed grasslands (GPP = 2708 g CO_2 m^{-2} $year^{-1}$) (Gilmanov *et al.*, 2010). Increased productivity in intensively managed grasslands is often as result of fertilizer application (Yue *et al.*, 2016). However, fertilizer application is discouraged in some grasslands due to environmental concerns which include protecting biodiversity and improving the quality of water courses. For example, agri-environmental schemes in the UK typically are targeted towards these environmental benefits but the consequences for ecosystem C flux have not been extensively explored.

Within the UK, upland grasslands are considered sensitive environments because they are unsuitable for crop production but have important conservation values as they contain species of plants that are scarce in Europe, and are breeding grounds for nationally scarce birds and amphibians (English Nature, 2001). These areas mainly occur at 250 – 300 m above sea level and are predominantly managed for livestock production (Stevens *et al.*, 2008) under both intensive management regimes, including fertilizer application to improve forage productivity, and extensive management regimes. The latter are prescribed by environmental stewardship schemes which aim to enhance biodiversity and usually entail planting of wild flowers, cutting for hay or silage only at specific times of the year, and no inorganic fertilizer applications.

Most C flux studies of the managed UK upland grasslands have so far been carried out in Scotland (e.g. Gilmanov *et al.*, 2007; Jones *et al.*, 2017; Quin *et al.*, 2015), and all reported negative NEE, indicating their C sequestration potential (Table A4.1). Methane flux was measured in only one study, which reported a negligible contribution to the net ecosystem C flux (Jones *et al.*, 2017). It is difficult to draw conclusions about the effect of management intensity on C flux in these upland grasslands, however, because of differences in soil type between extensively and intensively managed sites. NEE was lower on extensively managed grassland on organic soils (-161 g CO_2 m^{-2} $year^{-1}$; Quin *et al.*, 2015) compared to intensively managed grassland (grazed, fertilised and cut for silage) on mineral soils (NEE = -218 to -1324 g CO_2 m^{-2} $year^{-1}$; Gilmanov *et al.*, 2007; Jones *et al.*, 2017). In order to guide new agri-environment schemes designed to deliver multiple benefits, there is a need to isolate the effects of management from inherent environmental characteristics.

In this study, our aim was to examine the ecosystem CO_2 and CH_4 fluxes in upland grasslands in northern England: 1) at two locations underlain by different parent materials, and 2) under traditional hay meadow and conventional pasture (cut for silage or permanently grazed) management regimes. We hypothesize that: 1) all the grasslands will be a net C sink, with the grasslands under traditional hay meadow management gaining more C than the grasslands under conventional pasture, 2) grasslands of siliceous stone-derived soils will gain more C than the grasslands of soils formed from limestone, and 3) the flux of C in all the grasslands will be significantly influenced by microclimate.

4.2 Methodology

4.2.1 Study area

We selected two locations for this study, one in Nidderdale (54°09'N, 01°53'W) and the other in Ribblesdale (54°05'N, 02°16'W), at an altitude of approximately 300 m above sea level and a distance of about 20 km apart. Both locations are within the Yorkshire Dales, an upland area of the Pennines in northern England, UK. At each location, there are two contrasting grassland management regimes: traditional hay meadow managed under an agri-environment scheme (both locations) and conventional pasture cut for silage (Nidderdale) or permanent grass continuously grazed by sheep (Ribblesdale). The traditional hay meadows under agri-environment schemes are managed with the aim of restoring, protecting and enhancing biodiversity, whereas the conventional pastures are managed primarily for livestock production. The conventional pasture in Ribblesdale (permanent pasture) was neither fertilized nor cut, whereas the conventional pasture in Nidderdale (silage pasture) received inorganic N and additional P and K in poultry manure (Table 4.1). The hay meadow site in Nidderdale receives 6 t ha^{-1} $year^{-1}$ of farmyard manure (FYM), whereas the Ribblesdale hay meadow has not received application of FYM since 2001. The Nidderdale sites were limed to raise soil pH three years before the start of our flux measurements.

The area has a cool and wet climate with mean annual temperature of 7.4°C and mean annual rainfall of 1550 mm (1981-2010, recorded at Malham Tarn station located 6 km from Ribblesdale and 18 km from Nidderdale). The Nidderdale sites are characterized by stagnohumic gley soils (Humic Gleysol) and formed from clay drift with siliceous stone content, whereas the Ribblesdale sites are underlain by brown earth soils (Eutric Cambisol) formed from carboniferous limestone. The soil characteristics are shown in Table 4.2. The dominant grasses at both locations are *Holcus lanatus* and *Lolium perenne*, together contributing more than 50% of the vegetation cover. Other grasses and herbaceous species that are common at the Nidderdale sites are *Ranunculus repens* and *Trifolium repens*, whereas the Ribblesdale sites have *Anthoxanthum odoratum, Geranium sylvaticum, Poa trivialis, Festuca rubra*, *Deschampsia cespitosa* and *Alopecurus pratensis*.

Table 4.1: Management activities in the study area.

Site	Management history	Inorganic fertilizer application	Organic manure application	Liming	Livestock stocking rate/grazing intensity
Nidderdale hay meadow	Traditional hay meadow for over 150 years	none	Surface application of 6 t ha^{-1} $year^{-1}$ of FYM in May.	Surface application of 5 t ha^{-1} Mg limestone in 2010 and 5 t ha^{-1} Ca limestone in 2013	10-15 sheep ha^{-1} in the month of April and from September to the end of December. Higher stocking density in September when the field is used as a holding area
Nidderdale silage pasture	Rough pasture for over 50 years and utilized also for silage since 2008	Surface application of 50 kg N ha^{-1} $year^{-1}$ since 2014 as NH_4NO_3 or $(NH_4)_2SO_4$ in early June	Surface application of 6 t ha^{-1} $year^{-1}$ of FYM in May. 100 kg each of both phosphate and potash applied in early June 2016 as burnt poultry manure	Surface application of 5 t ha^{-1} Mg limestone in 2010 and 5 t ha^{-1} Ca limestone in 2013	15-20 sheep ha^{-1} in the month of April and from September to the end of December
Ribblesdale hay meadow	Traditional hay meadow for over 25 years	None since 1995. Annual dressing of 20 kg N ha^{-1} as NPK 20:10:10 from 1980 to 1995	Surface application of 4-6 t ha^{-1} $year^{-1}$ of FYM from 1990 to 2001	None since 1995. There was occasional annual dressing previously	10-15 sheep ha^{-1} in the months of April, September and October; intermittent grazing between January and March
Ribblesdale permanent pasture	Conventional pasture for over 25 years	None	None	None	5 sheep ha^{-1} all year round

FYM = farmyard manure, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium.

4.2.2 Soil sampling and analysis

Soils were sampled in May 2016, at the start of the gas monitoring period. Five 25 m^2 (5 m x 5 m) replicate plots were established in a single field of each management type and at each of the two locations for soil sampling. Within each of the plots, soil samples (0 – 15 cm depth) were collected from five points with a soil auger (5 cm diameter) and bulked into a composite sample. One additional sample was collected from each plot for bulk density determination. Samples were transported and stored at 4°C prior to analysis at the School of Geography, University of Leeds, UK. Nitrate – nitrogen (NO_3 – N) and ammonium – N (NH_4 – N) were extracted from the fresh soil samples with 1M potassium chloride (KCl) and analyzed within 24 hours using a Skalar SAN++ auto analyzer. Stone-free bulk density was determined by drying core samples at 105°C in an oven for 24 hours and dividing the weight of dry soil by the volume of the core occupied by the soil after correcting for stoniness (percentage of rock fragments greater than 2 mm in diameter; Poeplau *et al.,* 2017):

$$Bulk\ density\ = \frac{Mass\ of\ dry\ soil - Mass\ of\ stones}{Volume\ of\ soil - \left(\frac{Mass\ of\ stones}{Density\ of\ stones\ (2.6\ g\ cm^{-3})}\right)} \qquad 4.1$$

The remaining soil samples were air dried and passed through a 2 mm sieve, after visible roots and other plant materials were removed. The sieved samples (< 2 mm) were used for the determination of all other soil physico-chemical properties. Soil pH was measured in a soil suspension of 1:2.5 soil-deionized water ratio (Robertson *et al.,* 1999). Total C and N were measured by combustion in an elemental analyzer (Vario Micro Cube), after the soil samples were first ball milled and passed through a 0.50 mm sieve. The total C values were used as SOC because the soil samples were free from carbonates when tested with hydrochloric acid (HCl). Exchangeable basic cations (Ca, Mg, K, and Na) were extracted with 1 M ammonium acetate (Allen, 1989) and analyzed using a Thermo Scientific iCAP 7600 Duo ICP-OES Analyzer. For particle size analysis, soil samples were dispersed using 5% sodium hexametaphosphate and passed through 0.53 mm sieve to separate the sand particles; the clay fraction was then determined by pipette method and the silt fraction calculated by subtraction (Van Reeuwijk, 2002).

4.2.3 CO_2 and CH_4 flux measurements

Four square collars (dimension: 62 cm length × 62 cm width × 25 cm height) made of 4-mm thick polyvinyl chloride sheets (Figure 4.1) were installed 10 m apart in each field. This gave a total of 16 collars for the four fields studied. The collars were driven half-way into the soil and were left in place for three weeks before the start of gas measurements so as to minimize the effects of the disturbance caused during installation. We measured CO_2 and CH_4 fluxes for twelve months from June 2016 to May 2017. Gas samples were collected once per month between 1000 and 1600 hours. The frequency of gas measurement and sampling was limited due to the remoteness and restricted access to the sites. The gas measurements were separated into NEE, the CO_2 exchange measured under sunlight, and ER, the CO_2 exchange measured when sunlight was excluded by use of a shroud.

During each gas sampling, the pre-installed collars were fitted with a transparent chamber 50 cm in height with internal area similar to those of the pre-installed collars. The chamber was constructed of 3 mm thick acrylic sheet and was fitted with an axial fan for headspace air mixing, a sampling port, pressure equilibration gas bags, and thermo-hygro-barometer for measuring chamber temperature, relative humidity and pressure. A silicone sponge seal was used to ensure that the contact point between the base of the chamber and the top of the collar was airtight, in order to prevent the exchange of gases between the chamber and the surrounding environment.

To measure NEE, the CO_2 concentration in the chamber was measured every 20 seconds for a period of 120 seconds with a portable infrared gas analyser (IRGA; EGM-4, PP Systems, Amesbury, USA; measurement precision = 1%). To measure ER, the transparent chamber was removed from the collar immediately after taking the last NEE reading, vented for 60 seconds and covered with a reflective shroud to prevent sunlight penetration. The chamber with its fitted shroud was then placed over the collars. Gas samples were collected with a 20 ml plastic syringe after 60 seconds of placing the chamber on the collar and at 5-minute intervals for a period of 25 minutes. The collected gas samples were injected into 12 ml pre-evacuated glass vials (Labco Limited, Lampeter, United Kingdom) and transported to the laboratory. The gas in vials was subsequently analysed for CO_2 and CH_4 using a gas chromatograph (GC Systems, Agilent Technologies, 7890A) equipped with a flame ionisation detector (measurement error for CO_2 and CH_4 fluxes were 3.0% and 3.1% respectively). In order to ensure comparability between NEE and ER measurements, gas samples for analysis by gas chromatograph (GC) were collected simultaneously with IRGA CO_2 measurement in the first month of gas measurement. Paired t-test result showed that the average concentration of CO_2 measured with the IRGA (502±26 ppm) was not significantly different ($p = 0.81$) from the concentration of CO_2 measured with the GC (498±24 ppm). During each time of gas measurement or sample collection in the field, we also recorded chamber temperature, relative humidity and pressure (Comet Thermo-hygro-barometer) as well as photosynthetically active radiation – PAR (Skye Quantum sensor), and soil temperature and moisture (GS3 Decagon sensors under standard calibration, coupled to a handheld Procheck meter). Soil temperature and moisture were also monitored continuously using Decagon smart sensors (S-TMB-M006, S-SMD-M005) installed at 10 cm depth and connected to a HOBO micro station (H21-002) logging at 15-minute intervals. Daily air temperature, precipitation and solar radiation data for our sites, covering the duration of the flux measurements (May 2016 to May 2017) and used for modelling CO_2 and CH_4 fluxes, were provided by the UK Meteorological Office (http://www.metoffice.gov.uk). The weather data were provided as mean daily temperature, total daily precipitation, and total daily solar radiation. Only about 1% of the weather data were missing including: 2 days for precipitation; 3 days for air temperature; and 5 days for radiation. The daily weather data for the Ribblesdale site were measured at Malham Tarn station (6.00 km away) whereas the weather data for Nidderdale site were measured at Middlesmoor weather station (1.80 km away and recording since 2013).

The fluxes of CO_2 and CH_4 were calculated based on the rates of increase or decrease in their concentrations (Denmead, 2008):

$$F = \frac{V}{A}\frac{d\rho}{dt} \qquad 4.2$$

where F = flux density at the grassland surface (mg m^{-2} s^{-1}), V = headspace volume (m^3), A = internal area of collar (m^2), ρ = mass concentration of the gas in the chamber headspace (mg m^{-3}) and t = time (s). The CO_2 and CH_4 flux values in mg m^{-2} s^{-1} were converted to mg

m^{-2} day^{-1} by multiplying with 86400 (i.e. the number of seconds in a day). The gas fluxes were estimated as the slope of the linear regression of CO_2 and CH_4 concentrations against time, after air temperature and pressure were corrected to standard values. Flux values were recorded if the slope of the linear regression was significant ($p < 0.05$) and the coefficient of determination (R^2) was equal to or greater than 0.75. The mean fluxes for each field was calculated by averaging the fluxes from the four replicate chambers. We adopted the atmospheric sign convention which defines a negative NEE as a net C uptake by the grassland ecosystem whereas positive NEE indicates C loss to the atmosphere (Imer *et al.*, 2013).

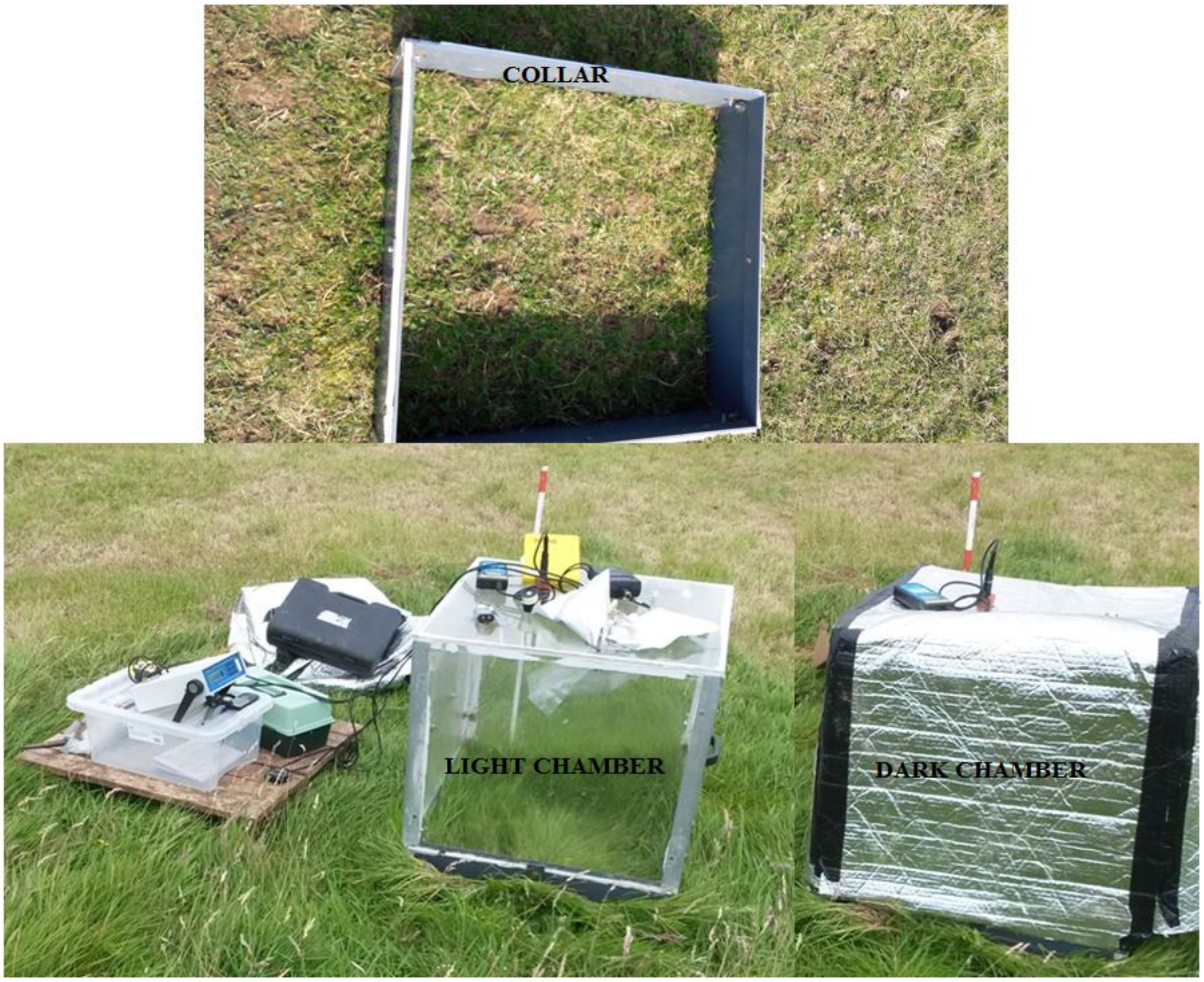

Figure 4.1: Collar and chambers used for C flux measurements.

4.2.4 Modelling annual CO_2 and CH_4 fluxes

To estimate annual CO_2 and CH_4 fluxes where there are gaps in C flux measurements, gap filling techniques based on the relationships between C fluxes and meteorological variables are used to fill the missing data in the annual time series (Moffat *et al.*, 2007). The approach commonly used for annual CO_2 flux is to model GPP and ER and then calculate NEE as the difference between the two (Moffat *et al.*, 2007). In this study, we modelled annual GPP and ER for each of our 16 collars and calculated NEE using the equation:

$$NEE = ER - GPP \qquad 4.3$$

4.2.4.1 Modelling annual GPP

To model GPP, we parameterised and compared the performance of two non-linear regression equations: a rectangular hyperbolic saturation curve (Thornley and Johnson, 1990; Equation 4.4) which has been widely used for modelling grasslands' GPP (e.g. Campbell *et al.*, 2015; Du *et al.*, 2014; Dyukarev, 2017; Elsgaard *et al.*, 2012; Huth *et al.*, 2017), and a multiplicative variant (Equation 4.5) which helps to account for subtle changes in moisture and temperature that may affect photosynthesis.

$$GPP = \frac{(\alpha \times PAR \times Gmax)}{(\alpha \times PAR + Gmax)} \qquad 4.4$$

$$GPP = \frac{Q \times PAR}{k + PAR} \times X_1 \times X_2 \qquad 4.5$$

where *Gmax* refers to the theoretical maximum rate of photosynthesis at infinite PAR (photosynthetic capacity), α is the initial slope of the hyperbolic equation (photosynthetic efficiency), *k* is the half-saturation constant, X_1 is soil temperature and X_2 is soil moisture. Soil temperature and moisture were used as multiplicative variables in Equation 4.5 because their daily data for the complete one year duration of our flux measurements were available. The two equations were fitted to recorded data from our sites and the 'Solver' function in Microsoft Office Excel (2010 version) was used to estimate the best fit parameters for *Gmax*, α, *Q* and *k* based on values that produced the smallest error term (sum of the squared difference between measured GPP and the GPP predicted by the curves; measured GPP was calculated with Equation 4.3 using measured NEE and ER). Using best fit parameters for each of the two equations, GPP values were predicted for the days with measured GPP. The predicted GPP from each equation were plotted against their corresponding measured GPP and a regression line fitted through the plots. The regression line fitted through the plot of GPP predicted with Equation 4.4 and measured GPP had relatively higher coefficients of determination ($R^2 = 0.81 – 0.95$) and lower root mean square errors (RMSE = 2.45 – 4.50) than that of Equation 4.5, with R^2 values of 0.42 – 0.65 and RMSE values ranging from 5.47 to 6.97 (e.g. Figures A4.1A and A4.1B). Equation 4.4 was therefore considered appropriate for modelling GPP.

The parameters of Equation 4.4 derived for each of the collars were applied to a set of daily PAR data (obtained from UK Met Office's solar radiation datasets). Daily GPP was generated for the period 1st June 2016 to 31st May 2017. The generated daily GPP data were summed to get the annual GPP.

4.2.4.2 Modelling annual ER

Most models of ER are based on exponential dependence on temperature (Moffat *et al.*, 2007) but the most commonly used is the Arrhenius model (Equation 4.6; e.g. Du *et al.*, 2014; Elsgaard *et al.*, 2012; Huth *et al.*, 2017; Jacobs *et al.*, 2007).

$$ER = R_{10}\, e^{E_0\left(\frac{1}{T_{ref} - T_0} - \frac{1}{T - T_0}\right)} \qquad 4.6$$

where T is air or soil temperature; R_{10} is ER rate at a reference temperature ($T_{ref} = 283.15$ K); T_0 is temperature when ER is zero, usually constrained to 227.13 K to avoid over-parameterisation (Elsgaard *et al.*, 2012; Huth *et al.*, 2017); E_0 is temperature sensitivity coefficient.

We parameterised the Arrhenius model using the same procedure for parameterising the equations in Section 4.2.4.1. Equation 4.6 was fitted to recorded soil temperature data from our fields and the 'Solver' function in Microsoft Office Excel (2010 version) was used to estimate the best fit parameters for R_{10} and E_0 based on values that produced the smallest error term. T_0 was constrained to 227.13 K. Using best fit parameters for Equation 4.6, ER values were predicted for the days with measured ER. The predicted ER were plotted against their corresponding measured ER and a regression line fitted through the plots. The regression line had a one-to-one slopes (0.984 – 1.003), high coefficients of determination (R^2 = 0.978 – 0.996) and very low RMSE values (0.26 – 0.57) (e.g. Figure A4.2). Equation 4.6 was judged to have performed very well in predicting measured ER and was therefore used for modelling ER. The parameters of the equation derived for each of the collars were applied to a set of daily soil temperature data recorded at our sites. Generated daily ER for one year were summed to get the annual ER.

4.2.4.3 Modelling annual CH_4 flux

Before estimating annual CH_4 flux, we tested the performance of simple linear and multiple linear regression functions in predicting measured CH_4 flux. For each of the collars, we plotted measured CH_4 flux against soil temperature and fitted a linear regression line through the plots. The simple linear regression based on soil temperature was extended to a multiple linear regression model by adding soil moisture. However, the regression models showed no significant relationships between measured CH_4 flux and either soil temperature or soil moisture (e.g. Figure A4.3 and Table A4.2).

As none of the measured environmental variables at our sites could be suitably used to predict CH_4 flux, a weighted total was used to estimate annual CH_4 fluxes based on the assumption that the methane flux did not change between one field visit and the next. This technique involved applying the flux value measured on a particular day to subsequent days until the next measurement, and so on. The daily CH_4 fluxes from 1st June 2016 to 31st May 2017 were then summed to get the annual flux. To calculate the GWP of our fields, annual methane fluxes were first converted to CO_2 equivalent emissions (CO_2-eq). The CO_2-eq was calculated by multiplying annual methane fluxes by a factor of 28, which is considered as the GWP of methane on a 100-year time scale (IPCC, 2013). The CO_2-eq and NEE values for each field were then added to get each field's GWP.

4.2.5 Statistical analysis

From the modelled daily C fluxes (see Section 4.2.4), we generated annual GPP, NEE, ER, CH_4 flux and GWP for each of our 16 collars. Mean annual fluxes for each field (n = 4 collars) were then calculated as the average (± standard error) of the four replicate collars. The normality and homogeneity of variance of the annual C flux data as well as all the measured soil parameters were established using Shapiro-Wilk normality test and Levene's test. One-way analysis of variance (ANOVA) was used to compare the mean annual C fluxes and soil properties between fields. The differences in mean values were separated using Tukey HSD post hoc test. Other statistical analyses related to modelling were as described in Section 4.2.4. All statistical analyses and modelling were carried out in SPSS Statistics (version 22) and Microsoft Excel (2010 version).

4.3 Results

4.3.1 Soil properties

The soils of the two locations were moderately acidic with pH ranging from 5.6 to 6.0 (Table 4.2). Soil bulk density was similar in the hay meadow fields (0.72 – 0.84 g cm^{-3}) and the conventional pasture fields (0.63 – 0.66 g cm^{-3}) of the two locations. Whereas the Nidderdale conventional pasture had a significantly greater SOC (8.0%) than the other fields (4.7 – 5.5%), the Ribblesdale conventional pasture had a significantly lower soil available nitrate (0.2 mg kg^{-1}) than all the other fields (0.7 – 1.2 mg kg^{-1}).

4.3.2 Seasonal air/soil temperature and moisture conditions

There were slight differences in the monthly patterns of air temperature and precipitation between the two locations from June 2016 to May 2017 (Figure 4.2), with the Ribblesdale fields being on average warmer (+0.7˚C) and in total drier (-49 mm or -5%) than the Nidderdale fields. The differences in air temperature had a marked seasonal effect, being greater during the summer. Air temperature at Ribblesdale was warmer than at Nidderdale fields by 1.0˚C in summer, and by 0.6˚C in all other seasons. Soil temperature in Nidderdale was cooler than Ribblesdale by about 1.0˚C in spring and summer but warmer in autumn (+2.8˚C). Also, the Nidderdale soils were wetter than those of Ribblesdale by 1 – 7% except during the summer when the Ribblesdale soils had greater soil moisture content (+0.8%). In Ribblesdale, the monthly moisture content of the two fields were similar with an average of 55%, whereas in Nidderdale the silage pasture had higher monthly soil moisture content (60%) than its hay meadow counterpart (53%).

4.3.3 Seasonal carbon fluxes

The seasonal pattern of C flux was similar across the grasslands, being high between spring and autumn, and low in winter (Figure 4.3). There was generally an uptake of CO_2 by the grasslands in all the seasons except in winter when there was a net CO_2 efflux from the ecosystem into the atmosphere (Figures 4.3a and 4.3c). The ER decreased progressively from the summer months to the winter months and started increasing in spring (Figure 4.3b). Methane flux was close to zero all year round except in the spring when there was relatively greater uptake by the grasslands (Figure 4.3d).

4.3.4 Annual carbon fluxes

The four grasslands were all net C sinks with annual NEE ranging from -2661 to -2748 g CO_2 m^{-2} in Nidderdale, and -1812 to -2257 g CO_2 m^{-2} in Ribblesdale (Figure 4.3; Table 4.3). The conventional pasture and hay meadow fields in Nidderdale had significantly greater NEE and lower ER than their Ribblesdale counterparts. The conventional pastures in the two locations were not significantly different in their GPP. In contrast, the Nidderdale hay meadow had a significantly greater GPP (4096 g CO_2 m^{-2} $year^{-1}$) than the Ribblesdale hay meadow (GPP = 3451 g CO_2 m^{-2} $year^{-1}$). There were no statistically significant differences in NEE, ER or GPP between the two fields in Nidderdale. In contrast, Ribblesdale permanent pasture had a significantly ($p < 0.05$) higher NEE (-2257 g CO_2 m^{-2} $year^{-1}$) and GPP (3920 g CO_2 m^{-2}) than its hay meadow counterpart (NEE = -1812 g CO_2 m^{-2} $year^{-1}$; GPP = 3451 g CO_2 m^{-2} $year^{-1}$). The four fields studied were CH_4 sinks, with no significant difference in their mean annual fluxes which ranged from -0.36 to -0.42 g CH_4 m^{-2} $year^{-1}$ in Nidderdale and -0.39 to -0.44 g CH_4 m^{-2} $year^{-1}$ in Ribblesdale. The fluxes of C in all the grasslands were dominated by CO_2 as CH_4 flux was generally very small (-0.36 to -0.44 g CH_4 m^{-2} $year^{-1}$).

When annual C fluxes were converted to CO_2-eq, the C removed from the atmosphere by the grasslands and its associated GWP were significantly different between the two locations, and between the two fields in Ribblesdale (Table 4.3). The atmospheric C removal was significantly lower in the Ribblesdale hay meadow with a GWP of -1822 g CO_2-eq m^{-2} $year^{-1}$ than in the Ribblesdale permanent pasture with a mean annual GWP of -2269 g CO_2-eq m^{-2} $year^{-1}$.

4.3.5 Relationship between carbon fluxes and soil variables

In addition to the strong control of PAR and soil temperature (Sections 2.4.1 and 2.4.2) on C flux, we explored potential relationships with other environmental variables. The only significant outcome was a linear positive relationship ($p = 0.03$, $R^2 = 0.95$) between GPP and available soil nitrate (Figure 4.4 and Figure A4.4; residual degree of freedom = 2).

Table 4.2: Mean ± standard error (n = 5) of soil physico-chemical properties of the study area.

Site	Soil texture	Bulk density (g cm^{-3})	SOC (%)	Total N (%)	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})
Nid. hay meadow	Sandy loam (65% sand, 11% clay)	0.84±0.03a	4.7±0.2a	0.4±0.02a	1.2±0.3a	1.5±0.45ab
Nid. silage pasture	Sandy loam (60% sand, 10% clay)	0.63±0.03b	8.0±0.5b	0.5±0.03b	1.2±0.1a	0.7±0.09a
Rib. hay meadow	Loam (50% sand, 7% clay)	0.72±0.03ab	5.5±0.2a	0.6±0.03b	0.2±0.1b	2.4±0.32b
Rib. Perm. pasture	Loam (50% sand, 14% clay)	0.66±0.05b	5.4±0.4a	0.6±0.04b	0.7±0.1a	1.6±0.21ab

Nid. = Nidderdale, Rib. Ribblesdale, Perm. = permanent, SOC = soil organic carbon, Ex = exchangeable, Ca = calcium, Mg = magnesium, K = potassium, Na = Sodium, N = nitrogen, NO_3 = nitrate, NH_4 = ammonium. Column means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

Table 4.2 (Continued): Mean ± standard error (n = 5) of soil physico-chemical properties of the study area

Site	Ex-Ca (mmol/kg)	Ex-Mg (mmol/kg)	Ex-K (mmol/kg)	Ex-Na (mmol/kg)	pH
Nid. hay meadow	41.4±0.9a	11.2±0.5a	2.0±0.19a	0.7±0.04a	6.0±0.04a
Nid. silage pasture	41.6±1.8a	10.0±0.4a	3.4±0.38b	1.5±0.21b	5.7±0.12b
Rib. hay meadow	36.0±0.5a	9.4±0.7a	0.7±0.09c	1.2±0.06b	5.6±0.03b
Rib. Perm. pasture	37.3±2.4a	11.9±1.0a	0.8±0.24c	1.1±0.05ab	5.8±0.10ab

Nid. = Nidderdale, Rib. Ribblesdale, Perm. = permanent, SOC = soil organic carbon, Ex = exchangeable, Ca = calcium, Mg = magnesium, K = potassium, Na = Sodium, N = nitrogen, NO_3 = nitrate, NH_4 = ammonium. Column means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

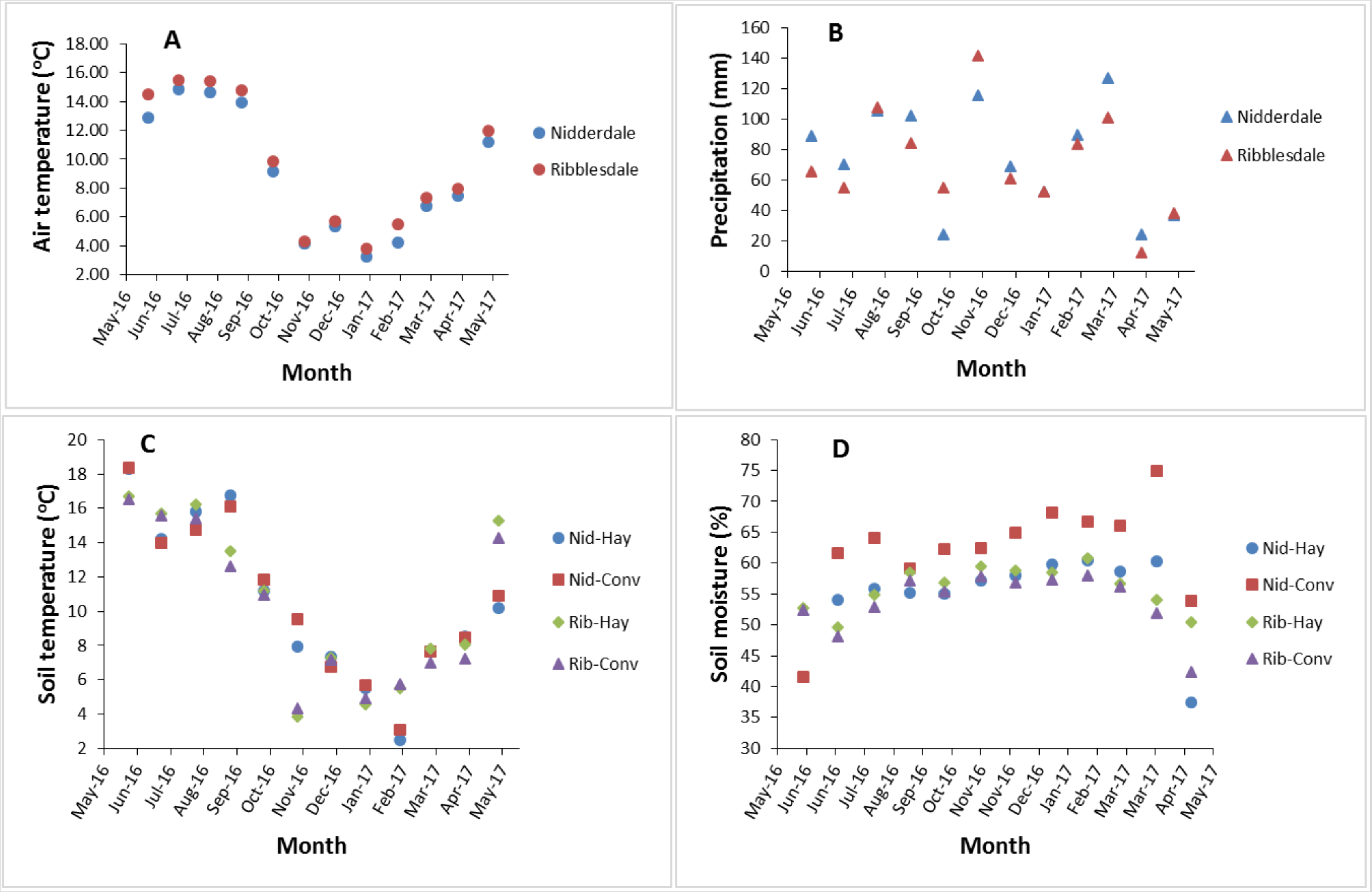

Figure 4.2: Monthly means of average daily air temperature (A), total monthly precipitation (B), and monthly mean soil temperature (C) and soil moisture (D) in Nidderdale (Nid) and Ribblesdale (Rib). Hay = hay meadow and Conv = conventional pasture.

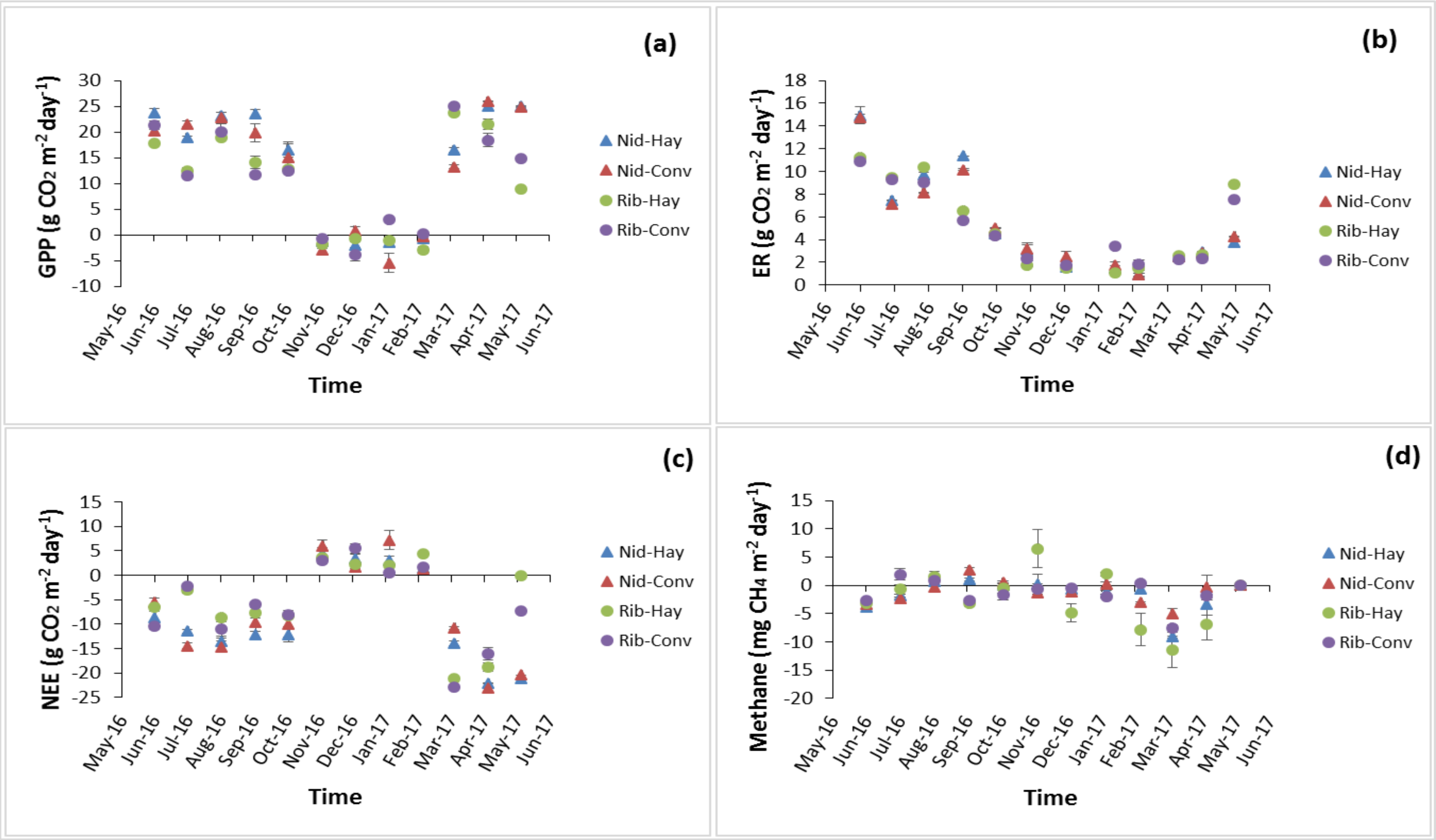

Figure 4.3: Measured C fluxes: (a) gross primary productivity – GPP, (b) ecosystem respiration – ER, (c) net ecosystem exchange – NEE and (d) methane in Nidderdale (Nid) and Ribblesdale (Rib). Hay = hay meadow and Conv = conventional pasture (silage in Nidderdale and permanent grass continuously grazed in Ribblesdale), coloured symbols represent mean fluxes measured per field and day of sampling (n = 4) and bars represent standard error.

Table 4.3: Annual ecosystem C flux and GWP with all values reported in g m^{-2} $year^{-1}$ (mean ± standard error, n = 4 per field).

Site	GPP	ER	NEE	Methane	Methane (CO_2-eq)	GWP
Nidderdale-hay meadow	4096±115a	1435±37a	-2661±133a	-0.42±0.18a	-12±5a	-2673±134a
Nidderdale-silage pasture	4213±66a	1465±53a	-2748±55a	-0.36±0.10a	-10±3a	-2758±58a
Ribblesdale-hay meadow	3451±82b	1639±13b	-1812±80b	-0.39±0.30a	-11±8a	-1822±85b
Ribblesdale-permanent pasture	3920±56a	1664±4b	-2257±59c	-0.44±0.19a	-12±5a	-2269±61c

GWP = global warming potential, GPP = gross primary productivity, NEE = net ecosystem exchange, ER = ecosystem respiration, eq = equivalent emissions. Column means followed by the same letter are not significantly different ($p > 0.05$) while those followed by different letters differ at $p < 0.05$.

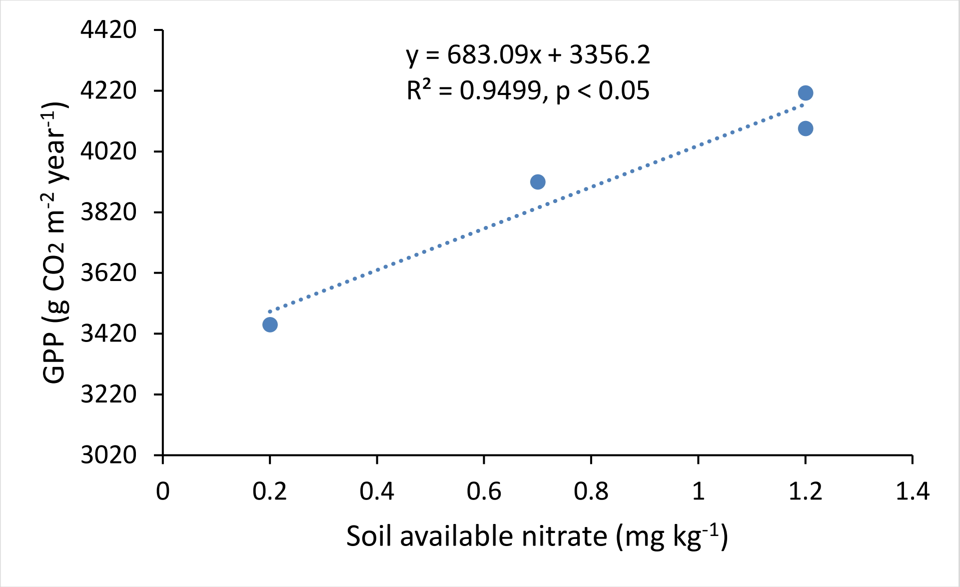

Figure 4.4: The relationship between annual gross primary productivity (GPP) and soil available nitrate.

4.4 Discussion

This study revealed that the upland grasslands in northern England were net atmospheric C sinks within the period considered, regardless of their differences in management and lithology, removing 1822 – 2269 g CO_2-eq m^{-2} year^{-1} in Ribblesdale and 2673 – 2758 g CO_2-eq m^{-2} year^{-1} in Nidderdale. This is partially consistent with our first hypothesis, although contrary to our expectation as the C gain in the hay meadows was not significantly greater than the C gain in the conventional pastures. This result agrees with many previous reports that temperate grasslands are a net atmospheric C sink. For example, managed grasslands in nine European countries including Italy, Denmark, France and Switzerland have been reported to remove atmospheric C; although the range in annual uptake of 22 to 1626 g CO_2 m^{-2} (Gilmanov *et al.*, 2007; Soussana *et al.*, 2007) is quite wide. An uptake of atmospheric C ranging from 258 – 1185 g C m^{-2} year^{-1} has also been reported in some Irish grasslands receiving inorganic N fertilizers and either being cut for hay, grazed by livestock or a combination of the two (Gilmanov *et al.*, 2007; Jaksic *et al.*, 2006; Peichl *et al.*, 2011). A much lower annual C uptake of 840 g C m^{-2} was measured in some managed Chinese grasslands (Li *et al.*, 2005). The C sink capacity of our grasslands, within the one year of our study, are greater than the values reported in other European grasslands, and other temperate grasslands outside Europe.

The reason for the greater C uptake in this study compared to other studies (e.g. Table A4.1) is attributable to lower C efflux, despite lower productivity. Our grasslands were less productive (GPP $\leq$ 4200 g C m^{-2} $year^{-1}$) than many of the grasslands reported in Table A4.1 with annual GPP of more than 4600 g C m^{-2} $year^{-1}$, but also had lower ER (< 1700 g C m^{-2} $year^{-1}$) than most of the grasslands in Table A4.1 (annual ER of 1803 – 5927 g C m^{-2} $year^{-1}$). The relatively cooler (mean annual air temperature, MAT = 7.4°C) and wetter (mean annual precipitation, MAP = 1550 mm) conditions at our sites may explain the lower C efflux compared to the areas reported in Table A4.1 with MAT and MAP that were mostly above 8.5°C and below 1200 mm respectively. Studies in the central Great Plains grassland in the USA (Rigge *et al.*, 2013) and the Temperate Steppe in Inner Mongolia (Hao *et al.*, 2013) have shown that increasing precipitation results in a significant reduction in C efflux. Temperature also is a controlling factor in C efflux in grasslands, as indicated in Equation 4.6 and reported in regional studies in the USA (Frank *et al.*, 2002) and China (Cui *et al.*, 2014; Fang *et al.*, 2012). The relatively higher C uptake by the grasslands in our study indicates a great potential for contributing to climate change mitigation, provided the C sink trend is sustained before the ecosystem reaches its C saturation point.

The C sequestration potential of our sites looks promising, but we acknowledge that inter-annual and inter-decadal variability in C fluxes occur and can only be assessed by continuous C flux measurements. Thus there is a danger of overestimating the C sink capacity of the sites. In the following sections, the implications of our findings, especially in relation to management activities and environmental factors are discussed.

4.4.1 Effect of management activities on ecosystem C flux

The C uptake of the Ribblesdale hay meadow was almost 20% lower than the Ribblesdale permanent pasture (1822 and 2269 g CO_2-eq m^{-2} $year^{-1}$, respectively), and 32 – 34% lower than the two fields in Nidderdale (2673 – 2758 g CO_2-eq m^{-2} $year^{-1}$). This was likely attributable to differences in available soil nitrogen considering the strong positive relationship observed between available soil nitrate and GPP (Figure 4.4). Levels of available soil nitrate in the Ribblesdale hay meadow (0.2 mg/kg NO_3 – N) were significantly lower compared to both the Ribblesdale permanent pasture (0.7 mg/kg NO_3 – N) and the Nidderdale fields (1.2 mg/kg NO_3 – N) (Table 4.2). These differences in soil available nitrate are likely due to subtle differences in management, and particularly in the different amounts of organic manure each site receives. The Nidderdale site receives FYM annually and the presence of livestock in the Ribblesdale permanent pasture all-year round makes for greater deposition of faeces and urine which enhance N mineralization (Frank *et al.*, 1994; Welker *et al.*, 2004; Yan *et al.*, 2016).

The addition of N to grasslands has been reported to induce an increase in net atmospheric CO_2 uptake in a number of field (Peichl *et al.*, 2011; Skinner, 2013) and mesocosm (Moinet *et al.*, 2016) experiments. In a temperate steppe grassland in northern China, for example, Niu *et al.* (2010) found an average of up to 27% increase in NEE due to addition of 100 kg N/ha/year, an increase that was equivalent in size to that seen for Ribblesdale permanent pasture and Nidderdale fields with relatively smaller N input (see Table 4.1). However, UK semi-natural landscapes such as upland grasslands also receive additional N input from atmospheric deposition, and this has been implicated in observed increases in net primary productivity (NPP) in recent years (Tipping *et al.*, 2017). However, the addition of N fertilizers to grasslands may sometimes result in a greater C loss than is gained. For example, Verburg *et al.* (2004) found that grassland net CO_2 emission was larger in response to N addition (88 kg N/ha) despite a doubling of biomass productivity, presumably because N limitation of microbial decomposition was alleviated by fertilisation. Similarly, the fertilization effects of faecal deposition in a grazed grassland may increase soil microbial activity, thereby increasing C efflux (Bardgett and Wardle, 2003). High N-induced soil C efflux mostly occurs when N fertilization enhances plants' deposition of fresh and readily decomposable substrates available to soil microbes (Fang *et al.*, 2012). In our sites, the influence of fertilisation on C uptake was greater than the influence on C efflux, which could be attributed to one of two mechanisms (Riggs and Hobbie, 2016): 1) a N-stimulated vegetation growth, and 2) an increase in C use efficiency of decomposer organisms, which happens when N addition alters the allocation of C acquired by microbes in favour of growth rather than respiration.

Although this study revealed that the fertilized grasslands had lower GWP potential, their contribution to climate change mitigation depends on their overall C and GHG balance. For example, the C gained by the ecosystem may be lost depending on the amount of C offtake by grazing animals when these are removed from the system, and when the vegetation is cut and removed. Also, fertilization may induce high N_2O emission, which is a more potent GHG than both CO_2 and CH_4 (Breitenbeck and Bremner, 1986). It is therefore important that subsequent research in these grasslands focus on the overall C inputs and C losses as well as N_2O emissions.

In contrast to a number of studies that demonstrate marked reduction in C uptake in grasslands under permanent grazing (Chen *et al.*, 2011; Haferkamp and MacNeil, 2004; Skinner, 2008), the continuously grazed Ribblesdale permanent pasture had higher C uptake than the Ribblesdale hay meadow. This was possibly because the Ribblesdale permanent pasture had lower stocking density (5 sheep ha^{-1}) than the Ribblesdale hay meadow (10 – 15 sheep ha^{-1}), and the months without grazing in the hay meadow could not offset the effects of its higher stocking density on C flux. Secondly, the loss in C resulting from the removal of vegetation during grazing is often over-compensated by the higher physiological activity and photosynthetic efficiency of younger leaves produced by regrowth compared to older leaves in temporarily or permanently ungrazed sites (Kjelgaard *et al.*, 2008; Owensby *et al.*, 2006).

The results of this study suggest that fertiliser application and low stocking density (e.g. 5 sheep ha^{-1} in the case of Ribblesdale) may be important in increasing upland grasslands' C sequestration. These initial findings need to be verified by controlled experimental field studies to inform policy decisions. In reality, the economic needs of farmers, the market demand for livestock production, and the desire to minimise unwanted environmental effects of added fertilisers (reduced biodiversity, water pollution) will all be factors in determining management choices. Our results have implications for agri-environment schemes as farmers under these schemes currently are discouraged from adding fertilizers in order to enhance biodiversity. Considering all these factors, the chances of achieving multiple ecosystem services in our grasslands such as climate change mitigation and increased biodiversity may be slim.

4.4.2 Influence of environmental factors on ecosystem C flux

We had expected a higher C gain in the Nidderdale fields whose soils were formed from siliceous parent material than the Ribblesdale fields with limestone-derived soils, due to potential differences in soil pH, texture, and moisture retention. Although the Nidderdale fields gained more C, there was no evidence that this was due to differences in soil type. Soil pH was similar across the two locations due to the liming of the Nidderdale fields, and whereas the two locations differed in their soil textural class, the soil moisture content of all the sites were well within the limit (≥ 40%; Borowik and Wyszkowska, 2016) considered favourable for biological activities. In addition to management effects that have been discussed in the preceding section, differences in C flux between the two locations may be partly due to subtle differences in climate rather than differences in soil type.

The higher ER in both the hay meadow and conventional pasture fields in Ribblesdale, compared to their Nidderdale counterparts, can therefore be attributed to the warmer (0.15 – 1.64 °C higher monthly temperature) conditions in Ribblesdale. Many studies (e.g. Cui *et al.,* 2014; Fang *et al.,* 2012; Frank *et al.,* 2002; Lin *et al.,* 2011; Wohlfahrt *et al.,* 2008) have shown that increasing soil temperature increases the respiratory C loss from the grassland ecosystem. This happens because temperature increases the speed of enzymatic reactions (Larcher, 2001) and stimulates microbial decomposition of organic matter (Jones *et al.,* 2006). In this study, ER ($r = 0.89$, $p = 0.004$) was more strongly related to temperature than GPP ($r = 0.06$, $p = 0.534$), suggesting a greater sensitivity of ER to warming. Hence, in a warmer future, the grasslands we studied and other temperate grasslands which are now C sinks may become a net C source. This is a particular concern for UK uplands considering that the seasonal pattern of temperature lapse rate with increasing altitude has changed, such that uplands are experiencing relatively milder winters (Holden and Rose, 2011). Climate manipulation experiments in which the effects of projected climate change can be rigorously tested are therefore needed to improve our understanding and inform appropriate management policy decisions that aim to increase C sequestration in these grasslands in the future.

4.5 Conclusion

The upland grasslands investigated were all atmospheric C sinks, removing 1822 – 2758 g CO_2-eq m^{-2} $year^{-1}$. This C sink is larger than those reported for other European grasslands due to low ER. The Ribblesdale hay meadow had the lowest net C uptake, which was attributed to low available soil N due to the absence of N fertilization. This has important implication for agri-environment schemes where farmers are encouraged not to add fertilizers to enhance biodiversity, thereby putting in doubt the possibility of mitigating climate change and at the same time enhancing biodiversity. The Ribblesdale sites had higher ER values than their Nidderdale counterparts, and this was mostly attributed to warmer conditions (0.15 – 1.64°C higher monthly temperature) prevailing in Ribblesdale, demonstrating that local-scale climate fluctuations can have a significant effect on C flux. Fertilizer application and temperature were the most important factors affecting C flux in the studied sites. Due to the potential effects of increased N fertilization on the emission of other GHGs, it is recommended that further research should consider the overall GHG balance of the grasslands, and that a focus on inter-annual and decadal variability should be a priority considering the potential strength of upland grasslands as GHG sinks. It is also recommended that further research be conducted on the response of upland grasslands C fluxes to predicted changes in temperature and moisture regimes.

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Chapter 5: Negative effects of climate change on upland grassland productivity and carbon fluxes are not attenuated by nitrogen status

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Abstract

Effects of climate change on managed grassland C fluxes and biomass production are not well understood. In this study, we investigated the individual and interactive effects of experimental warming (+3°C above ambient summer daily range of 9-12°C), supplemental precipitation (333 mm +15%) and drought (333 mm -23%) on plant biomass, MBC, NEE and DOC flux in soil cores from two upland grasslands of different soil N status (0.54% and 0.37%) in the UK. After one month of acclimation to ambient summer temperature and precipitation, five replicate cores of each treatment were subjected to three months of experimental warming, drought and supplemental precipitation, based on the projected regional summer climate by the end of the 21st Century, in a fully factorial design. NEE and DOC flux were measured throughout the experimental duration, alongside other environmental variables including soil temperature and moisture. Plant biomass and MBC were determined at the end of the experiment. Results showed that warming plus drought resulted in a significant decline in belowground plant biomass (-29 to -37%), aboveground plant biomass (-35 to -77%) and NEE (-13 to -29%), regardless of the N status of the soil. Supplemental precipitation could not reverse the negative effects of warming on the net ecosystem C uptake and plant biomass production. This was attributed to physiological stress imposed by warming which suggests that future summer climate will reduce the C sink capacity of the grasslands. Due to the low moisture retention observed in this study, and to verify our findings, it is recommended that future experiments aimed at measuring soil C dynamics under climate change should be carried out under field conditions. Longer term experiments are recommended to account for seasonal and annual variability, and adaptive changes in biota.

Keywords: climate change; warming; drought; carbon flux; grassland management; biomass productivity.

5.1 Introduction

Ecosystem sequestration of atmospheric C in terrestrial ecosystems is a function of the balance between C uptake by plants (GPP) and C loss via processes such as ER and leaching, and these processes are sensitive to climate, particularly precipitation and temperature (Albaladejo *et al*., 2013; Bellamy *et al*., 2005; Rees *et al*., 2005). The global climate is warming and precipitation patterns are also changing, with regional differences reported (IPCC, 2013; Jenkins *et al.*, 2009). Rising temperature and changing precipitation is also expected throughout the 21^{st} Century, with global mean surface temperature projected to increase by 0.3 – 0.7°C by 2035 and 1.7 – 4.8°C by 2100, relative to 1986 – 2005 baseline (IPCC, 2014).

Climate change is expected to exert significant effects on terrestrial ecosystem C pools and their fluxes including plant biomass (Hartmann and Niklaus, 2012), microbial biomass (Rui *et al.,* 2011), NEE (the difference between GPP and ER; De Boeck *et al.,* 2007), and the leaching of DOC (Hagedorn and Joos, 2014), with possible feedbacks to climate change. A very useful method for investigating the response of these ecosystem processes to climate change is experimental manipulation of climate variables such as temperature and precipitation (Bloor *et al.,* 2010). Climate manipulation experiments have been set up to explore the responses of plant productivity and ecosystem C fluxes to climate change in different ecosystems (e.g. Allison and Treseder, 2008; Baldwin *et al.,* 2014; Beierkuhnlein *et al.,* 2011). A synthesis of data from 85 of these experimental studies (Wu *et al.,* 2011) in different ecosystems across the globe including forests, shrublands and grasslands shows that: 1) warming without manipulated precipitation enhanced both ecosystem photosynthesis and respiration with no effect on net C uptake, 2) increased precipitation without warming enhanced both ecosystem photosynthesis and respiration with an overall increase in net C uptake, and 3) decreased precipitation without warming suppressed both ecosystem photosynthesis and respiration with an overall decrease in net C uptake. Another recent synthesis of results of about l60 climate manipulation experiments in different ecosystems also revealed that warming increased both soil C input and loss with no significant effect on net C pool, whereas increased precipitation stimulated soil C input, and drought suppressed it (Ni *et al.,* 2017). Thus, the balance of evidence indicates that altered precipitation patterns have greater control on net soil C store than warming.

Past climate manipulation experiments in different terrestrial ecosystems (see reviews by Liu *et al.,* 2016; Ni *et al.,* 2017; Wu *et al.,* 2011) have usually explored the individual effects of warming, supplemental precipitation and drought on net C uptake. The interactive effects of changing temperature and precipitation on C cycling have rarely been examined and remain poorly understood (Lei *et al.,* 2016), although global meta-analyses indicate that the interactive effects of warming and altered precipitation differ from their simple additive effects (Ni *et al.,* 2017; Wu *et al.,* 2011). This means that adding up the reported individual effects of manipulated climate variables will not give the true response of the ecosystem. The effects of experimental climate change on the terrestrial C cycle are also confounded by other site-specific characteristics and management practices such as vegetation type (Beierkuhnlein *et al.,* 2011; Bloor and Bardgett, 2012; Miranda *et al.,* 2009) and fertilizer application (Jonasson *et al.,* 1999), especially nitrogen (N) fertilizers (Dukes *et al.,* 2005). For example, Graham *et al.* (2014) found that the addition of 50 kg N/ha increased the positive effect of warming on soil C efflux by 12% in a New Zealand grassland. Thus, for an improved understanding of the response of managed ecosystems to climate change, there is need for multifactorial experiments where the interactive effects of management, warming, supplemental precipitation and drought will be investigated.

Grasslands store a large amount (34%) of the global terrestrial C and provide important ecosystem services such as climate change mitigation and forage for livestock production (White *et al.,* 2000). In European grasslands, which are already net C sinks (Chang *et al.,* 2015), the majority of the climate manipulation experiments (see Tables A5.1 and A5.2) have focused on the effects of climate change on AGB and soil respiration (SR), and less attention has been given to other important C cycling processes such as NEE and DOC leaching. In published studies (Table A5.1) the main effects were: 1) warming alone stimulated both an increase and a decrease in AGB; 2) increased precipitation alone resulted in both an increase and a decrease in AGB; 3) drought alone decreased AGB and SR; and 4) both positive and negative interactive effects were observed when warming was combined with either increased precipitation or drought. The lack of definitive pattern of response to climate change in these studies may be partly due to pre-existing differences in grassland productivity. This is possible because less productive grasslands tend to be more resistant to climate perturbations (Grime *et al.*, 2000). Fertilizer is widely used to improve grassland productivity (Yue *et al.*, 2016), hence it is likely to be a major confounding factor in interpreting climate change effects. Whereas some grasslands are fertilised to increase vegetation biomass for livestock production, fertilizer application is discouraged in other grasslands due to environmental concerns such as protecting and enhancing biodiversity, or protecting water courses from pollution (Reed *et al.*, 2009). The effects of climate change on fertilized and unfertilized grasslands need to be investigated. This will help inform future management decisions for targeted outcomes in the face of climate change.

The need to investigate climate change effects on managed grasslands is a particular concern for UK uplands. These areas mainly occur at 250 – 300 m above sea level and have witnessed changes in climate that are much greater than in the lowlands (House *et al.*, 2010). For example, between 1961 and 2000, minimum temperatures increased more than maximum temperatures in the uplands, whereas there was no difference in the changes between minimum and maximum temperatures in the lowlands (Burt and Holden, 2010). Morecroft *et al.* (2009) also found that temperature trends between 1993 and 2007 differed between upland and lowland sites in the UK, with an average temperature increase of 1.2°C in the uplands and 0.7°C in the lowlands. The UK upland grasslands are considered sensitive environments and have important conservation values because they contain species of plants that are scarce in Europe, and are breeding grounds for nationally rare birds and amphibians (English Nature, 2001). These upland grasslands are predominantly managed for livestock production (Stevens *et al.*, 2008) under both extensive management regimes with no fertilizer application, and more intensive management regimes with fertilizer application to improve forage productivity for silage and grazing.

Climate manipulation studies in the UK upland grasslands are few (e.g. Briones *et al.*, 2009; Grime *et al.*, 2008), and how grasslands under different management regimes might respond to warming and altered precipitation has not been considered. Briones *et al.* (2009) investigated the response of an unimproved acid grassland in Scotland to a 2-year soil warming (+3.5°C) and found an increase in both ER and root biomass, and a decrease in AGB. Similarly, Grime *et al.* (2008) studied the response of an unfertilized grassland in Buxton (northern England) to 13 years of winter warming (+3.0°C), supplemental summer precipitation (+26%) and summer drought (-77%). Warming, increased precipitation and drought both separately and in combination, had little effect on the ecosystem, however, there was a reduction in AGB under all the treatments (Grime *et al.,* 2008). It remains to be known how fertilised upland grasslands respond to climate change. Specifically, there is a dearth of information on the effects of warming and altered precipitation on the net C uptake by plants and soil microbes as well as DOC flux.

The main aim of this study was to assess the individual and interactive effects of experimental warming, supplemental precipitation and drought on plant biomass, MBC, NEE, and DOC flux in two upland grassland fields of different soil N status in northern England. Earlier work (Eze *et al.,* 2018a) showed that these grasslands store large amount of soil organic C (SOC, 59 – 101 Mg ha^{-1}) and that about 70% of these C stock is occluded within the soil mineral mass. Whereas Eze *et al.* (2018a) identified the relative size of current SOC fractions, in this study we are investigating how the main fluxes and labile ecosystem C pools respond to short term climate manipulation. Changes in the bulk soil C stock or the relatively stable mineral-occluded C can only be detected in the long term (e.g. 10 – 100 years) (Smith, 2004). In contrast, changes in the less stable C pools such as the DOC, MBC and biomass accumulation, and C fluxes such as NEE, which are important indicators of changing environmental conditions, can be detected in the short term. The study was therefore based on the following hypotheses: 1) experimental warming and drought will separately and in combination reduce plant biomass, NEE, MBC, and DOC flux, 2) supplemental precipitation alone and experimental warming plus supplemental precipitation will stimulate higher plant biomass, NEE, MBC, and DOC flux, 3) the effects of experimental warming and altered precipitation will be greater in the high N field.

5.2 Methodology

5.2.1 Study area

Soil mesocosms from upland grasslands in Nidderdale (54°09'N, 01°53'W; Figure 5.1), northern England, were used for this study. Detailed site characteristics and management information are as described by Eze *et al.* (2018a). Briefly, the site has cool and wet climate with MAT of 7.4°C and MAP of 1550 mm (1981 – 2010). The soil is a sandy loam stagnohumic gley (Humic Gleysol in the World Reference Base), formed from clay drift with siliceous stone content. We selected two fields for this experiment. One field (high N field, HNF) receives inorganic N addition and has a significantly higher soil N (0.54%) and organic C stock (101 Mg/ha) than the low N field (LNF, total soil N = 0.37%, soil organic C stock = 59 Mg/ha). The herbaceous species common to the two fields are *Ranunculus repens* L. and *Trifolium repens* L. Other grasses in the HNF are *Holcus lanatus* L.*,* *Agrostis gigantea* Roth*,* and *Anthoxanthum odoratum* L.*,* whereas the LNF has *Lolium perenne* L.

5.2.2 Soil core sampling, experimental design and setup

We extracted 30 soil cores of 15 cm depth with intact vegetation (approx. 5 cm in height) from each of the two fields using un-plasticized polyvinyl chloride pipes (PVCu pipes, 11 cm diameter and 20 cm length). One end of each core was bevelled for ease of driving into the soil and the soil cores were extracted on the 31st of May 2017. After extraction, the cores were immediately taken to an environmental room in the School of Geography, University of Leeds.

The experiment consisted of six climate treatments in a fully factorial design of two temperature (ambient air temperature, T_0; ambient air temperature plus 3°C, T_3) and three precipitation (ambient precipitation, P_0; ambient precipitation plus 15%, P_{+15}; ambient precipitation minus 23%, P_{-23}) conditions (Table 5.1). The six treatment combinations were applied to five replicated cores of each field type (LNF and HNF), resulting in a total of 60 experimental cores. The 30-year (1981 – 2010) mean summer (June – August) precipitation and temperature (recorded at Malham Tarn station located 18 km from the site) were used as the P_0 and T_0. The warming (T_3) and altered precipitation (P_{+15} and P_{-23}) treatments were based on the most recent UK climate projection (UKCP09) for the latter part of the 21st Century (2070 – 2100) (http://ukclimateprojections.metoffice.gov.uk/). The changes projected for our site under medium emission scenarios and 50% probability are: 3.0°C increase in mean winter temperature, 3.3°C increase in mean summer temperature, 15% increase in mean winter precipitation and 23% decrease in mean summer precipitation. We chose the medium emission and 50% probability scenario because it represents the change in climate that is likely not to be exceeded (Jenkins *et al.*, 2009). Although summer condition was the focus of our study, we included supplemental precipitation (P_{+15}) in order to account for possible uncertainties in the prediction of seasonal precipitation, which is known to be highly variable (Jenkins *et al.*, 2009).

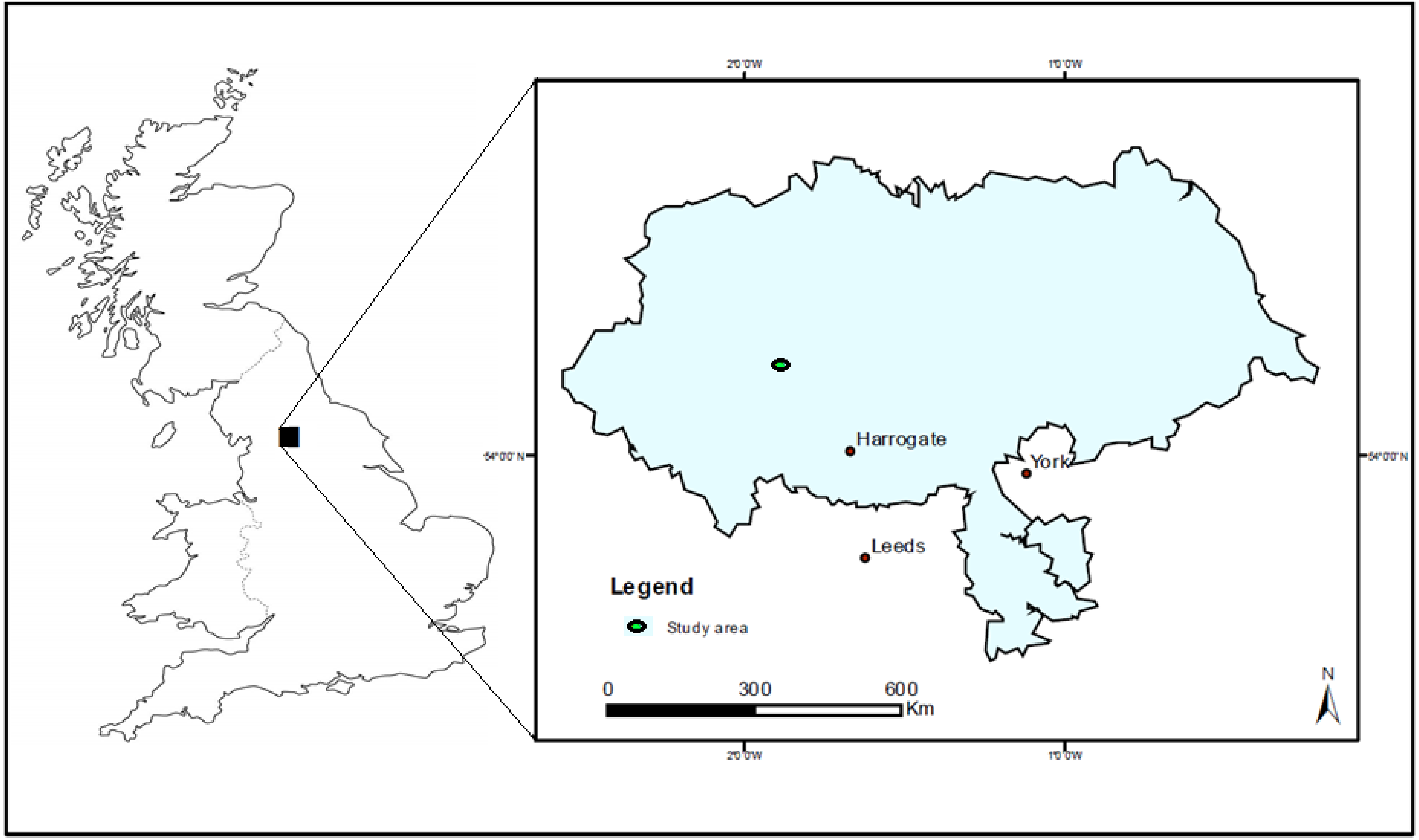

Figure 5.1: Map of North Yorkshire in the UK showing the area where soil cores were extracted.

Table 5.1: Experimental design based on observed 30-year mean summer (June – August) air temperature and precipitation and their 2070 – 2100 projection.

	Ambient summer temperature, T_0 (minimum = 9˚C, maximum = 16˚C)	Ambient summer temperature plus 3˚C, T_3, (minimum = 12˚C, maximum = 19˚C)
Low nitrogen field (LNF)	Ambient summer precipitation (P_0, 333 mm)	Ambient summer precipitation (P_0, 333 mm)
	Drought (P_{-23}, 256 mm)	Drought (P_{-23}, 256 mm)
	Supplemental precipitation (P_{+15}, 383 mm)	Supplemental precipitation (P_{+15}, 383 mm)
High nitrogen field (HNF)	Ambient precipitation (P_0, 333 mm)	Ambient precipitation (P_0, 333 mm)
	Drought (P_{-23}, 256 mm)	Drought (P_{-23}, 256 mm)
	Supplemental precipitation (P_{+15}, 383 mm)	Supplemental precipitation (P_{+15}, 383 mm)

The environmental room was set to T_0 with diurnal changes from a minimum of 9˚C at 0200 hr to a maximum of 16˚C at 1200 hr. In order to apply the two temperature treatments (T_0 and T_3) within the environmental room, we constructed two environmental chambers, ECs, (200 cm height × 116 cm width) with wooden frames (Rough sawn timber, 25 mm thickness × 38 mm width) and Celotex insulation board (Celotex TB4025 General Application Insulation Board, 25 mm thickness × 1200 mm width). Each of the ECs was fitted with an LED growth light (Heliospectra RX30), an Ecoheater (1500 mm Slimeline Greenhouse & Shed Heater HHT315 – 190W), two axial fans (Sunon Maglev DC 12V HA series) and a humidity/temperature data logger (EXTECH RH10). The Heliospectra lights were programmed to simulate 16 hours of light and 8 hours of darkness, which corresponds to the average summer photoperiod of the site. Light intensity was set to simulate diurnal variation, increasing from the first light-hour (40 µmol m^{-2} s^{-1}) to a maximum intensity at the ninth light-hour (850 µmol m^{-2} s^{-1}) and then decreasing until it becomes dark. The photosynthetically active radiation (PAR) recorded on-site from June 2016 to May 2017 was used to set the diurnal light intensity in the ECs. After setting up the lights, the environmental room temperature settings were adjusted to correct the increase in temperature resulting from heats generated by the lamps.

After the ECs were set up, wooden platforms (15 cm height) were placed on the floors of the chambers to support the soil cores. A total of 30 lysimeters, which served as the base for the soil cores, were arranged in five rows on the wooden platform. The lysimeters were made with 110 mm double socket PVCu couplers and plugs, fitted with high-density polyethylene funnels (Azlon FWC104, 300 ml capacity). The top of the funnels were covered with 1 mm nylon mesh to keep soil particles in the cores intact and allow easy drainage of water. A transparent plastic container (125 ml) was placed directly underneath each lysimeter to collect leachates from the soil cores. To avoid any leachate loss, a silicone tubing (Food Grade Flexible Hose, 11 mm diameter) was connected to each funnel to direct leachates into the containers. Five replicate soil cores for each precipitation treatment and from each of the two Nidderdale fields (HNF and LNF) were placed on the lysimeters in a completely randomized design, resulting in 30 cores in each EC. One core from each replicated treatment combination was fitted with soil moisture and temperature sensors (5TM Decagon sensors) coupled to Arduino loggers (Kona 328 Arduino Uno compatible development board).

5.2.3 Treatments and C flux measurement

The soil cores were kept under control conditions (i.e. P_0 and T_0) for 30 days (1st to 30th June 2017) for acclimation. Under the control condition, we applied 7.4 mm of artificial rain to the cores every other day. The frequency of rainfall was based on the average monthly summer rain-days for the site which was 14. In the absence of rainfall chemistry data for the site, the artificial rain was prepared in the laboratory using a published rainfall chemistry data from a long-term monitoring site (Moor House – Upper Teesdale; 54°41' N, 2°23' W) about 60 km away (Table A5.3). After the acclimation period, the temperature of one EC was raised by 3°C using the Ecoheater that had been fitted in the chamber. One-third of the soil cores in each chamber were still maintained under P_0 treatment, whereas the remaining two-thirds of the soil cores were spilt between P_{+15} and P_{-23} treatments. For the P_{+15} and P_{-23} treatments, 8.5 mm of synthetic rain was applied every other day. However, the drought treatment (P_{-23}) received rain only in the first and the third months of the treatments. The one month of no rain in the P_{-23} treatment was used to simulate an extreme drought event of 30 days which is similar to the 100-year recurrent drought event in both the UK (Bloor and Bardgett, 2012) and Germany (Mirzaei *et al.*, 2008). The treatments lasted for three months (1st July to 30th September 2017) after the acclimation period. Throughout the treatment period, we recorded air temperature and relative humidity (EXTECH RH10 data loggers) at 1 hr intervals, and soil moisture and temperature (5TM Decagon sensors) at 15 minutes intervals. We also collected leachates from the cores weekly, which were analyzed for DOC using thermal oxidation analysis of aqueous samples (Analytik Jena Multi N/C 2100).

Weekly measurements of CO_2 and CH_4 fluxes were made throughout the experiment using an ultra-portable GHG analyzer (Los Gatos Research UPGHGA, model 915-0011, California). The gas measurements were separated into NEE, the CO_2 exchange measured under light, and ER, the CO_2 exchange measured when light was excluded. During each gas measurement, the soil cores were fitted with cylindrical covers 34 cm in height with internal diameter similar to those of the soil cores. The cover used for NEE measurement was constructed of transparent PVC pipe (110 mm diameter; > 90% light transmission), whereas opaque terracotta drainage pipe (110 mm diameter; 0% light transmission) was used to construct the cover used for ER measurement. The top of each cover was fitted with an inlet and outlet gas tubing connected to the gas analyser, an axial fan for headspace air mixing and pressure equilibration gas bags. A pipe coupling (110 mm diameter) was attached to the base of each cover to ensure that it fitted tightly on the soil cores and prevent exchange of gases with the surrounding environment.

CO_2 and CH_4 fluxes were measured from 48 (4 per treatment) out of the 60 soil cores as the remaining 12 cores had soil moisture and temperature sensors in them and could not be fitted with the covers. To measure NEE, the core was fitted with the transparent cover after being connected to the gas analyser. After 60 seconds of placing the transparent cover on the core, the CO_2 concentration in the cover was measured continuously for a period of 120 seconds. To measure ER, the transparent cover was removed from the core immediately after taking the last NEE reading, the core was vented for 60 seconds and fitted with the opaque cover connected to the gas analyser. After 60 seconds of placing the opaque cover on the core, the CO_2 and CH_4 concentrations in the cover were measured continuously for a period of 120 seconds. During each gas measurement, a respirator (3M 7501 Silicone half mask respirator) connected to the outside of the environmental room was worn to remove any exhaled CO_2. This was done to prevent an increase in the CO_2 concentration in the environmental room. We also recorded temperature and pressure of the chamber environment (Comet Thermo-hygro-barometer), and PAR (Skye Quantum sensor) during each gas flux measurement.

The fluxes of CO_2 and CH_4 were calculated based on the rates of increase or decrease in their concentrations (Denmead, 2008):

$$F = \frac{V}{A}\frac{d\rho}{dt} \qquad 5.1$$

where F = flux density at the soil core surface (mg m^{-2} s^{-1}), V = headspace volume (m^3), A = internal area of soil core (m^2), ρ = mass concentration of the gas in the cylindrical cover headspace (mg m^{-3}) and t = time (s). The CO_2 and CH_4 flux values in mg m^{-2} s^{-1} were converted to mg m^{-2} day^{-1} by multiplying with 86400 (i.e. the number of seconds in a day). The gas fluxes were estimated as the slope of the linear regression of CO_2 and CH_4 concentrations against time, after air temperature and pressure were corrected to standard values. Flux values were recorded if the slope of the linear regression was significant ($p < 0.05$) and the coefficient of determination (R^2) was equal to or greater than 0.75. The mean fluxes for each treatment combination were calculated by averaging the fluxes from four replicate soil cores. We adopted the atmospheric sign convention which defines a negative NEE as a net C uptake by the soil cores whereas positive NEE indicates C loss to the atmosphere (Imer *et al.*, 2013).

5.2.4 Modelling CO_2 and CH_4 fluxes

After we calculated the fluxes of CO_2 and CH_4 using Equation 5.1, the CH_4 fluxes were not significantly different from zero, hence only CO_2 fluxes were modelled. To estimate CO_2 flux for the treatment period (i.e. 3 months), we filled gaps in the daily time series based on the relationships between CO_2 flux and meteorological variables. The GPP and ER for the treatment period were modelled for each of the 48 soil cores and NEE was calculated using Equation 5.2.

$$NEE = ER - GPP \qquad 5.2$$

5.2.4.1 Modelling GPP

A rectangular hyperbolic saturation curve (Equation 5.3) is widely used for modelling GPP in grasslands (e.g. Dyukarev, 2017; Elsgaard *et al.*, 2012; Huth *et al.*, 2017).

$$GPP = \frac{(\alpha \times PAR \times Gmax)}{(\alpha \times PAR + Gmax)} \qquad 5.3$$

where *Gmax* refers to the theoretical maximum rate of photosynthesis at infinite PAR (photosynthetic capacity), α is the initial slope of the hyperbolic equation (photosynthetic efficiency). To model GPP, we tested the performance of Equation 5.3 for predicting the GPP we measured (measured GPP was calculated with Equation 5.2 using measured NEE and ER). Equation 5.3 was fitted to PAR data recorded in the environmental chambers and the 'Solver' function in Microsoft Office Excel (2010 version) was used to estimate the best fit parameters for α and *Gmax* based on values that produced the smallest error term (sum of the squared difference between measured GPP and the GPP predicted by the curve). Using best fit parameters for the equation, GPP values were predicted for the days with measured GPP. The predicted GPP were plotted against their corresponding measured GPP and a regression line fitted through the plots (e.g. Figure A5.1). The regression line had a relatively high slope (0.77) and coefficient of determination (R^2 = 0.97), hence, Equation 5.3 was considered appropriate for modelling GPP. The parameters of Equation 5.33 derived for each of the 48 soil cores were then applied to a daily set of PAR data recorded in the environmental chambers. Daily GPP was generated for the period 1st July 2017 to 30th September 2017 and summed.

5.2.4.2 Modelling ER

To model ER, we tested the performance of two exponential models (Equations 5.4 and 5.5) that have been used to model ER in grassland studies (e.g. Du *et al.*, 2014; Elsgaard *et al.*, 2012; Huth *et al.*, 2017).

Arrhenius model: $$ER = R_{10}\, e^{E_0\left(\frac{1}{T_{ref}-T_0} - \frac{1}{T-T_0}\right)} \qquad 5.4$$

Multiplicative model: $$ER = ae^{bT} \cdot SWC^{c} \qquad 5.5$$

where T is soil temperature; R_{10} is ER rate at a reference temperature (T_{ref} = 283.15 K); T_0 is temperature when ER is zero, usually constrained to 227.13 K to avoid over-parameterisation (Elsgaard *et al.*, 2012; Huth *et al.*, 2017); E_0 is temperature sensitivity coefficient; *SWC* is soil volumetric water content; *a, b,* and *c,* are fitting parameters. We parameterised the two models using the same procedure for parameterising the rectangular hyperbolic curve in Section 5.2.4.1. The equations were fitted to soil temperature and moisture data recorded for our soil cores and the 'Solver' function in Microsoft Office Excel (2010 version) was used to estimate the best fit parameters for R_{10}, E_0, *a, b,* and *c* based on values that produced the smallest error term. Using best fit parameters for the two equations, ER values were predicted for the days with measured

ER. The predicted ER were plotted against their corresponding measured ER and a regression line fitted through the plots.

Compared to the Arrhenius model, the multiplicative model performed better in predicting measured ER as shown by its higher slope and coefficient of determination (Figures A5.2A and A5.2B). However, we found that the slope of the multiplicative model was always less than 0.5. We therefore tested the performance of a multiple linear regression based on soil temperature and soil moisture (Equation 5.6). The ER predicted by Equation 5.6 were plotted against their corresponding measured ER and a regression line fitted through the plot. The regression line had almost one-to-one slope (0.98), with a lower root mean square error (RMSE = 0.32) than that of the multiplicative model (slope = 0.38; RMSE = 1.43; Figure A5.2). We therefore used Equation 5.6 for modelling ER. The coefficients of Equation 5.6 derived for each of our soil cores were applied to the daily set of soil temperature and moisture data that we recorded. Generated daily ER from 1st July 2017 to 30th September 2017 were summed to get the ER for the treatment period.

$$ER = aT + bM + c \qquad 5.6$$

where T is the soil temperature; M is the soil moisture content; a and b are slopes; c is the intercept.

5.2.5 Plant biomass measurement and soil analysis

At the end of the treatment period, the experiment was dismantled and the cores were taken to the laboratory for biomass and soil analysis. Aboveground biomass (AGB) of each core was cut with scissors to the soil surface and was sorted into dead biomass (brown and yellow, DAGB) and live biomass (yellowish green and green, LAGB). The intact bulk soil in each core was carefully extruded into an empty semi-cylindrical core of similar diameter as the cores used for the experiment. The extruded soil was split vertically into two equal halves using a knife with serrated edge. Half of the soil removed after the split was used for the determination of soil properties such as MBC. The other intact half in the semi-cylindrical core was used for root or belowground biomass (BGB) determination. The soil sample for BGB determination was washed on sieves of different mesh sizes (50 µm to 2 mm) to separate roots from the soil, and the roots were collected on the sieves. The roots and AGB were dried in an oven at 65°C for 48 hours and then weighed.

Soil MBC was determined using the chloroform fumigation-extraction method (Vance *et al.*, 1987) after visible roots were removed. Fresh soil samples (25 g dry weight equivalent) from each of the 60 soil cores were fumigated for 24 hours with ethanol-free chloroform ($CHCl_3$). After fumigation, residual $CHCl_3$ vapour was removed by repeated evacuations using water aspirator pump and two-stage rotary oil pump. Additional un-fumigated soil samples of similar weight as the fumigated samples were used as controls. Fumigated and un-fumigated samples were extracted with 75 ml of 0.5M K_2SO_4 (1:3 soil/extractant ratio). The samples were shaken for 1 hour and filtered through Whatman GF 934-AH filter paper. The soluble C in the fumigated and un-fumigated samples were then determined using an aqueous analyzer (Analytik Jena Multi N/C 2100). MBC was calculated as the difference between extractable C in the fumigated and un-fumigated samples divided by a conversion factor, K_{EC}, of 0.35.

5.2.6 Data analysis

For each soil core, we calculated the total DOC flux and NEE for the three months of the experiment. Total NEE was calculated from modelled GPP and ER as explained in Section 5.2.4. Total DOC flux was calculated by summing the weekly fluxes obtained by multiplying the concentration of DOC in analyzed aliquots by the volume of leachates collected. The normality and homogeneity of variance of the total DOC and NEE as well as MBC and plant biomass were established using Shapiro-Wilk normality test and Levene’s test. For each of the soil core type (LNF and HNF), a split-plot analysis of variance (ANOVA; with temperature as main plot factor and precipitation as sub-plot factor) was used to compare the means of DOC, NEE, MBC and plant biomass between treatments. The differences in mean values were separated using Tukey HSD post hoc test. Multivariate ANOVA (MANOVA) could not be used to assess the overall effect of each treatment on the C pools (MBC and plant biomass) and fluxes (DOC and NEE) due to multicollinearity. Moderate correlations between dependent variables are ideal for MANOVA, however, the correlations between our dependent variables were either too low ($r < 0.3$) or too high ($r > 0.8$). Other statistical analyses related to modelling were as described in Section 5.2.4, and all statistical analyses and modelling were carried out in Microsoft Excel (2010 version) and SPSS Statistics (version 22).

5.3 Results

5.3.1 Effect of warming and altered precipitation on C pools

Plant biomass and MBC were significantly affected by experimental warming and altered precipitation (Tables 5.2 and 5.3). The response of plant biomass to the climate manipulation was similar across HNF and LNF cores, whereas the response of MBC to climate manipulation differed between the two core types (Figure 5.2). There was a warming-induced decline in BGB and TAGB by -26% and -14% in the LNF, and -29% and -22% in the HNF respectively. Across both LNF and HNF, warming plus drought led to significant reductions (-73% and -77%) in LAGB and a significant increase (+192% and +215%) in DAGB. Drought resulted in a significant increase (+75) in the DAGB and a significant decline (-63%) in the MBC of the LNF but had no significant effects on any of the C pools in the HNF. Warming alone and warming plus supplemental precipitation significantly enhanced MBC by +125% and +171% respectively in the HNF, with no significant effects in the LNF.

5.3.2 Effects of warming and altered precipitation on C fluxes

The NEE was the only component of the C flux that was significantly affected by climate manipulation as the DOC flux was not significantly affected in either HNF or LNF cores (Table 5.3). All the temperature and precipitation treatments, except supplemental precipitation in the LNF, resulted in the decline of NEE. In the LNF, supplemental precipitation led to a significant increase (+103%) in NEE, whereas in the HNF, drought significantly reduced NEE by -66% (Figure 5.3). In the LNF and HNF, warming plus drought reduced NEE by -29% and -13% respectively. Similarly, warming plus supplemental precipitation reduced NEE by -14% and -24% respectively in the LNF and HNF, although this was not statistically significant.

Table 5.2: Mean ± standard error of plant biomass, microbial biomass carbon, net ecosystem exchange (NEE) and dissolved organic carbon after experimental warming and altered precipitation in cores of low (LNF) and high (HNF) nitrogen status (n = 5 for all parameters except NEE with n = 4).

Soil core	Temperature	Precipitation	Belowground biomass (g/m^2)	Dead aboveground biomass (g/m^2)	Live aboveground biomass (g/m^2)	Total aboveground biomass (g/m^2)	Microbial biomass carbon (mg/kg)	NEE* (g CO_2/m^2)	Dissolved organic carbon* (mg C)
LNF	Ambient	Ambient	1403±53	394±93	2330±200	2724±227	163±32	-1256±436	5±1
		Drought	1804±157	690±106	2166±154	2856±145	60±19	-1238±90	5±1
		Supplemental	1585±238	392±120	2809±156	3201±224	76±26	-2546±574	9±3
	Warming	Ambient	1038±152	326±63	2020±171	2346±130	151±28	-967±121	4±1
		Drought	1001±126	1151±89	623±231	1775±183	85±55	-891±94	4±0
		Supplemental	1056±185	404±78	1969±208	2373±165	239±31	-1078±151	4±0
HNF	Ambient	Ambient	1752±314	487±93	2387±73	2874±139	83±32	-1312±320	7±1
		Drought	2012±413	656±91	2145±111	2801±126	49±23	-451±117	11±3
		Supplemental	1988±224	439±53	2662±262	3100±258	91±42	-816±104	8±1
	Warming	Ambient	1241±144	484±67	1751±310	2234±252	187±49	-1120±103	5±1
		Drought	1110±235	1533±74	548±118	2081±187	72±23	-1147±111	5±1
		Supplemental	1292±449	491±113	1602±207	2093±137	225±75	-997±135	8±1

* = sum of the daily values for the experimental period (1st July to 30th September 2017).

Table 5.3: Split-plot ANOVA result showing the individual and interactive effects of temperature and precipitation on plant biomass, microbial biomass C (MBC), net ecosystem CO_2 exchange (NEE) and dissolved organic C (DOC) flux in cores of low (LNF) and high (HNF) nitrogen status (n = 5 for all parameters except NEE with n = 4).

Soil core	Source of variation	BGB		DAGB		LAGB		TAGB		MBC		NEE*		DOC*	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Low N	Temp.	14.18	0.01	1.93	0.20	40.38	<0.01	33.50	<0.01	3.42	0.10	4.26	0.09	2.76	0.15
	PPT.	0.74	0.49	32.55	<0.01	14.24	<0.01	3.00	0.08	5.16	0.02	7.48	0.01	2.00	0.18
	Temp. × PPT	1.09	0.36	6.76	0.01	4.97	0.02	1.71	0.21	4.51	0.03	5.55	0.02	1.76	0.21
High N	Temp.	8.60	0.02	13.24	0.01	42.04	<0.01	20.40	<0.01	7.88	0.02	2.90	0.14	5.04	0.07
	PPT.	0.10	0.91	48.80	<0.01	9.92	<0.01	0.41	0.67	2.27	0.14	3.29	0.07	0.91	0.43
	Temp. × PPT	0.18	0.84	23.15	<0.01	3.02	0.08	0.59	0.57	0.72	0.50	3.48	0.06	1.54	0.25

Temp. = temperature, PPT. = precipitation, BGB = belowground biomass, DAGB = dead aboveground biomass, LAGB = live aboveground biomass, TAGB = total aboveground biomass, * = sum of three months' daily fluxes, Sig. = significant value at 5% probability level. Bold font indicates significant effect at $p < 0.05$.

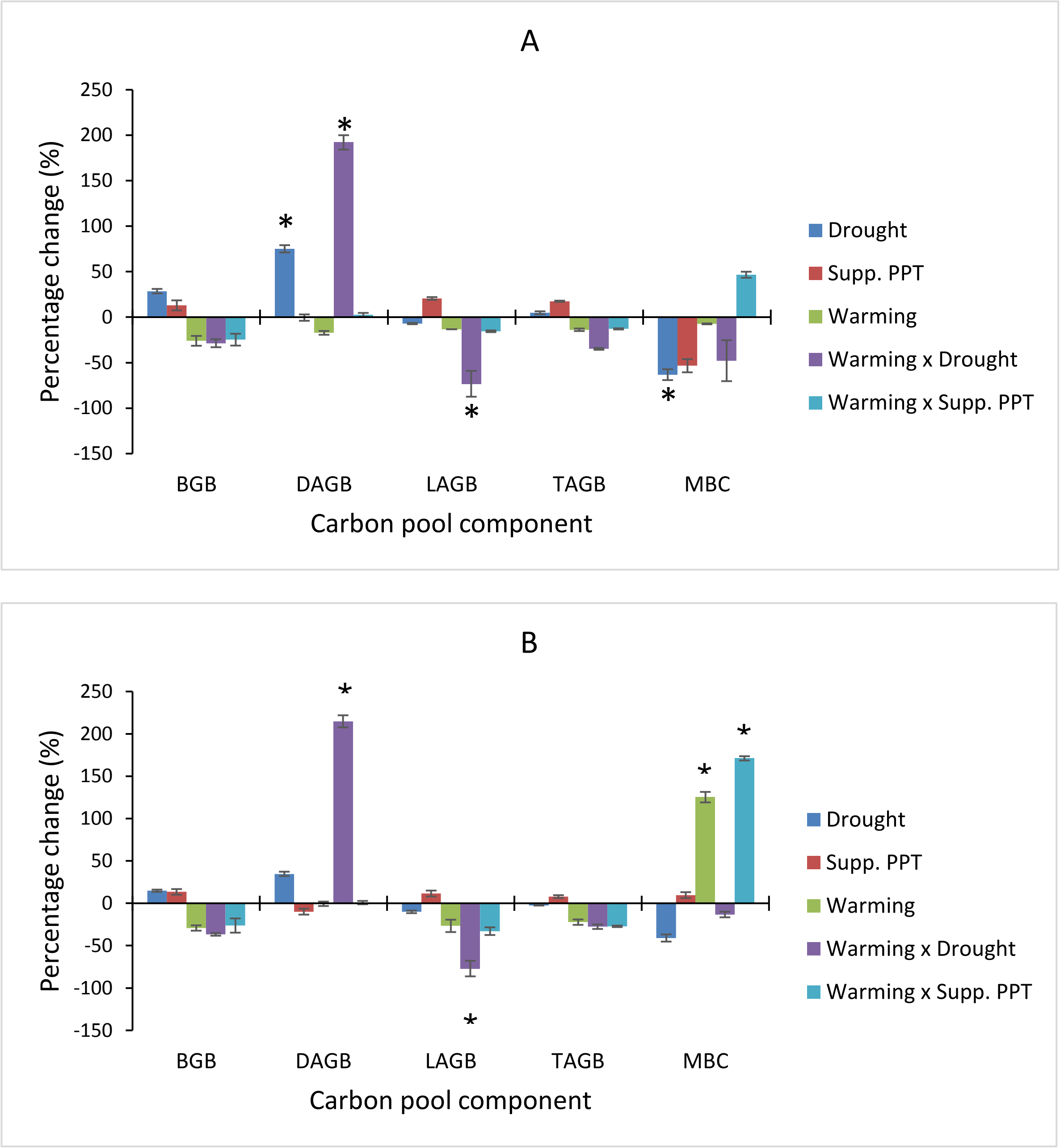

Figure 5.2: The effects of warming, drought, supplemental precipitation (Supp. PPT) and their interactions on plant biomass (belowground – BGB, dead aboveground – DAGB, live aboveground – LAGB, total aboveground – TAGB) and microbial biomass C (MBC) in cores of (A) low nitrogen and (B) high nitrogen status (n = 5). Bars with asterisks represent significant change at 5% probability level in the C pool component of soil cores subjected to altered climate treatment compared to cores with ambient climate treatment. Error bars represent standard error (n = 5).

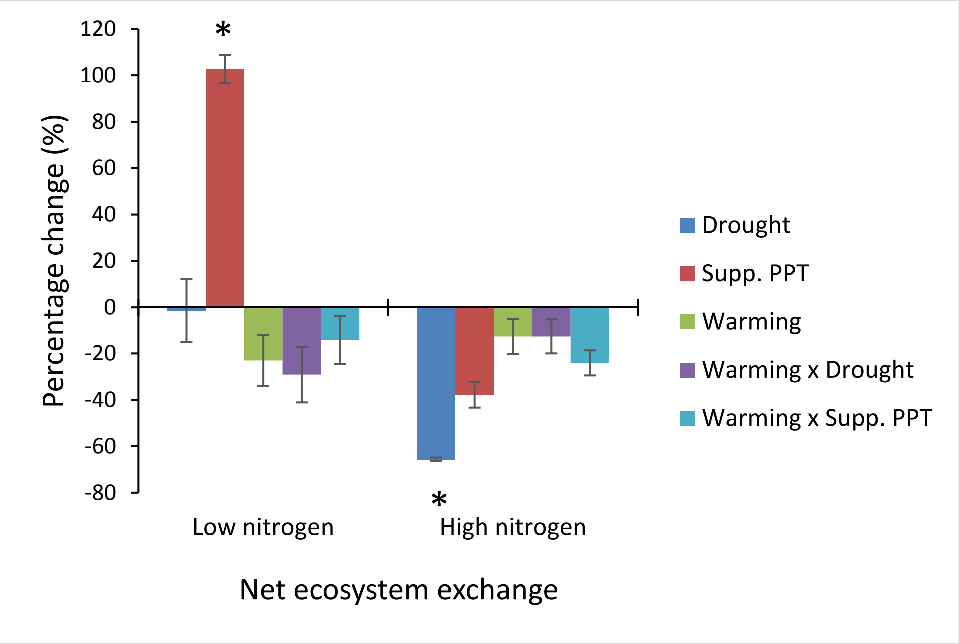

Figure 5.3: The effects of warming, drought, supplemental precipitation (Supp. PPT) and their interactions on the net ecosystem CO_2 exchange (NEE) in the cores of low nitrogen and high nitrogen status (n = 4). Bars with asterisks represent significant change at 5% probability level in NEE of soil cores subjected to altered climate treatment compared to cores with ambient climate treatment. Error bars represent standard error (n = 4).

5.4 Discussion

A combination of warming and drought resulted in a significant reduction in plant biomass production regardless of the N status of the soil cores. This is consistent with our first hypothesis where we expected a decline in C uptake and storage due to warming and drought. The reduction in aboveground plant biomass (-28 to -35% or -793 to -949 g m^{-2}) and NEE (-13 to -29% or -165 to -365 g CO_2 m^{-2}) resulting from warming plus drought in our study is consistent with the findings of previous experimental climate change studies in the UK (Table A5.1), and a Europe-wide C flux study carried out after a heat wave and drought in 2003 (Ciais *et al.*, 2005). Our results are also consistent with the results of modelling studies (e.g. Thornley and Cannell, 1997) which indicate a decrease in net ecosystem C uptake and plant biomass production under warming scenarios in temperate grasslands.

In temperate ecosystems where plant growth is limited by low temperature (Wingler and Hennessy, 2016), particularly in upland environments, one would expect an increase in biomass production under elevated temperature. However, the results from our study indicate that the response of the temperate grassland ecosystem to rising temperature will depend on the level of stress imposed by a combination of warming and altered precipitation. Detailed interactive effects of warming and drought as well as warming and supplemental precipitation, and the implications of these for future grassland management are discussed in the following sections.

5.4.1 Effects of warming and drought on net ecosystem C uptake and plant biomass production

Consistent with our findings, previous studies have reported that drought conditions under experimental warming suppressed plant growth and reduced MBC (Ganjurjay *et al.,* 2016; Liu *et al.,* 2009). In extreme cases, the survival of some species of plant can be threatened by a combination of warming and drought (Xu *et al.,* 2014). These negative interactive effects of warming and drought are attributable to physiological stress resulting from severe water deficits. By the end of the drought period, the soil cores in our study under warming and drought had less than half (5 – 12%) of the moisture content of soils under field conditions at our site during summer months (54 – 64%; Eze *et al.,* 2018b), indicating that severe physiological stress was the likely cause of increased plant death (Figure 5.2). Severe moisture deficit has been shown to cause low stomatal conductance (Sanaullah *et al.,* 2011), a decrease in the mobility of nutrients and reduced microbial access to substrates (Fuchsluege *et al.,* 2014). These impose stress on plants and soil microbes resulting in a significant reduction in C uptake by plants (Hasibeder *et al.,* 2015), plant biomass (Hartmann and Niklaus, 2012), microbial activities (Liu *et al.,* 2009), MBC (Shi *et al.,* 2012), and an increase in the death of plants and microbes (Sanaullah *et al.*, 2011).

Our findings suggest that under future summer climate change, particularly elevated temperature and drought, the C sink capacity of the grasslands we studied will be reduced, with likely similar effect on other temperate upland grasslands. This may lead to the ecosystem switching from being a net C sink to a net C source. It is however important to be cautious in extrapolating our results because other factors may dampen the negative effects reported. For example, the suppression of photosynthetic activity and plant growth, and increased senescence and mortality, resulting from the combination of warming and drought has been found to be partly alleviated by elevated atmospheric CO_2 in greenhouse studies (Van De Velde *et al.,* 2015; Xu *et al.,* 2014). In some grassland field manipulative experiments where climate change was simulated, elevated atmospheric CO_2 alongside experimental warming led to an increase in the net C uptake by plants (Mueller *et al.,* 2016; Ryan *et al.,* 2017). In addition, we considered only summer conditions thus reflecting the current short growing season for mid-to-high latitude grasslands, but the effects of climate extremes such as drought can be carried over to other seasons (Niu *et al.,* 2014). Under climate change, not only are lagged changes possible but the growing season may also be extended (Cleland *et al.,* 2007; Xia *et al.,* 2014) due to increased temperatures at either end of the summer. Hence multifactorial manipulative field experiments are needed that extend beyond the current growing season length, in order to determine the extent of ecosystem recovery from and resilience to multi-factor climatic stress (Van De Velde *et al.,* 2015).

5.4.2 Effects of warming and supplemental precipitation on net ecosystem C uptake and plant biomass productivity

The lack of significant effects of warming plus supplemental precipitation on net ecosystem C uptake and plant biomass production is surprising. Although warming is known to increase the loss of water from grassland ecosystems via increased evapotranspiration, supplemental precipitation recharges the ecosystem's water storage, often leading to a decrease in soil temperature (e.g. -0.5°C in this study) due to the high heat capacity of water (Lal and Shukla, 2004; Liu *et al.*, 2009). As a result, we had expected supplemental precipitation in this study to remove any water limitation imposed by elevated temperature, and at the same time minimize any direct warming-induced stress on plants and microbes possibly leading to increased C uptake. This was however not the case as only MBC increased under warming and supplemental precipitation whereas net C uptake and plant biomass productivity showed a non-significant decline. This indicates that supplemental precipitation may not sufficiently offset the negative effects of elevated temperature under severe warming-induced plant stress, at least in the short term.

Seasonal precipitation in the UK is highly variable (Jenkins *et al.*, 2009) and this makes the prediction of seasonal distribution of precipitation difficult. Although summer precipitation in the grasslands we studied is expected to decline in the future, including supplemental precipitation in this study helped to show that even if precipitation should increase in the future, it will not significantly reverse the negative effects of summer warming on net C uptake. A limitation of our work, however, is the short duration imposed by limited resources. The results reported here might have been different if the experiment was conducted for a longer period of time rather than restricted to growing season duration.

Another aspect of our study that needs to be considered in extrapolating the results is the possibility that drainage from the cores was enhanced because of their open end. This most likely added to the stress caused by warming-induced evapotranspiration on both plants and soil microbes. Water storage capacity in the soil cores (15 cm in depth) was likely to be less than found in field conditions where soil depth averages 20 cm from the surface down to the subsoil. Even when the top soil layer (e.g. the rooting zone) is dry, water stored deeper in the profile is made available to plants via capillary action (Vervoort and Van Der Zee, 2008). Also, plant roots grow more extensively during moisture stress which makes it possible for greater soil volume to be explored for water (Briones *et al.*, 2009). These sources of water to plants were absent from our mesocosm experiment. It is also possible that CO_2 might have been lost from the open bottom of the soil cores.

5.4.3 Implication of experimental warming and altered precipitation for future land management

Mesocosms from the two sites investigated here were both responsive to warming and altered precipitation, suggesting that the level of N addition in the HNF did not increase its sensitivity to climate change. The effects of altered precipitation on some components of the C cycle in the LNF and HNF differed slightly. For example, drought led to a significant increase in the death of AGB and a significant decline in MBC in the LNF, whereas in the HNF, drought significantly reduced NEE. The significant drought-induced death of AGB in the LNF may be connected to the presence of *Lolium perenne* L., which has been shown to be sensitive to drought (Aper *et al.*, 2014). The death of plants in the LNF might subsequently have affected the rate of rhizodeposition thereby leading to the reduction in MBC (Bloor *et al.*, 2018). On the other hand, the drought-induced reduction in the NEE of the HNF could be attributed to the reduction in GPP and an increase in ER (Figure A5.3), which limited the net C uptake by the plants. Despite the slight differences in the responses of LNF and HNF to altered precipitation, LNF and HNF had similar responses to both drought and supplemental precipitation when combined with warming. These findings were contrary to our third hypothesis where we expected greater effects of climate manipulation on the HNF that receives inorganic N addition. In an earlier UK study, Grime *et al.* (2000) reported that fertile grasslands with fast growing species were more sensitive to experimental climate change than less fertile and more matured grasslands. The site studied by Grime *et al.* (2000) that was considered fertile and more productive was a successional grassland converted from arable land, whereas our fields have been managed for grazing animals for over 100 years. Hence, the differences in sensitivity to climate change reported by Grime *et al.* (2000) represented land use change and possibly a more extreme comparison than the relatively small differences in N application reported here, representing typical upland grazing management.

Our results indicate that soil water management during the growing season may present a greater challenge to C uptake and biomass production than nutrient addition by the end of the 21st Century. However, as explained earlier, the level of soil moisture loss recorded in our mesocosm experiment is likely to be higher than observed in field conditions. Our study was also short term making it difficult to account for adaptive changes in plant and microbial species' physiology and community composition (Grime *et al.,* 2000), which are detectable in the long term. Multi-year climate manipulative studies under field conditions are called for to inform and devise appropriate strategies for future grassland management that will be climate-smart.

5.5 Conclusion

A combination of warmer summer (+3°C) and drought (-23% precipitation) conditions led to a significant decline in the net ecosystem C uptake (-13 to -29%) and plant biomass production (-29 to -37% belowground biomass and -35 to -77% aboveground biomass) after three months of experimental manipulation. Supplemental summer precipitation (+15% precipitation) could not reverse the negative effects of warming on the net ecosystem C uptake and plant biomass production. Mesocosms representing grassland sites with differing N status (0.54% and 0.37%) were equally responsive to the experimental climate treatments, suggesting that differences in N management may not have significant influence on the response of the grasslands to climate change. The negative effects of the experimental climate change on the net ecosystem C uptake was attributed to physiological stress resulting from severe soil moisture deficits. However, in addition to water losses resulting warming-induced evapotranspiration, the reduction in soil moisture might be partially attributable to open-ended mesocosms used in this experiment. The cores have less volume and capacity to retain water than is possible in the field. This led to the recommendation that future experimental climate change studies in the upland grasslands should be carried out in the field. Also, due to the short term nature of our experiment, it is further recommended that long term studies should be conducted to account for adaptive changes in plant and soil microbial species that may result from changing temperature and precipitation regimes.

Acknowledgement

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Chapter 6: Critical discussion and conclusions

SOC sequestration is recognized as the most cost-effective strategy for reducing atmospheric C, which is a major factor responsible for global warming and climate change (Lal, 2013). Storing C in soils as a component of SOM also helps to improve soil health, crop and forage productivity as well as environmental quality (Lal, 2016). Soils differ in their C storage capacity due to the influence of climate, management practices, aboveground vegetation type and soil characteristics (Olsson *et al.,* 2009). In order to enhance SOC storage, it is necessary that the most appropriate management activities are chosen for specific soil-land use-ecoregion combinations. This thesis investigated the impact of climate change and land management on C dynamics of British upland grassland soils, with the aim of providing an evidence base to support new agricultural land management policies that will be sustainable. This section draws together the findings in Chapters 2 – 5, and integrates the findings from independent chapters in order to discuss implications for future management of the UK upland grasslands. Limitations of the study and directions for future work are also discussed.

6.1 Major research findings

The main aim of this thesis was to assess the impacts of climate change and management regimes on the C dynamics of UK upland grassland soils. The observational and experimental studies carried out to achieve this overall aim were driven by four research questions and specific objectives. Here, I present a summary of the major research findings that provided answers to the research questions, and which indicate the achievement of the research objectives.

6.1.1 The response of SOC stock to management activities in global grasslands

The aim of Chapter 2 was to investigate the effects of inorganic fertilizers, liming and grazing on SOC stock of grasslands in different climate settings (thesis objective 1). The results showed an overall significant reduction (-8.5%) in the stock of SOC in global managed grasslands relative to unmanaged grasslands. Grazing significantly reduced SOC stock by -15%, liming resulted in a non-significant increase (+5.8%) whereas fertilizer application significantly increased SOC stock by +6.7%. The management effects on SOC stock was greatest in the tropics (-22.4%) and least in the temperate zone (-4.5%). The smallest effect of management on SOC stock was found when MAT was in the range of 0 – 5.0°C (-4.9%). The effects were greater at MAT below 0°C (-8.4%) and above 5.0°C (-17.2%). The management effect was only significant when MAP was below 600 mm (-11.7%). In the temperate zone, the overall negative effect of management practices on SOC stock decreased with increasing MAT and MAP.

6.1.2 The effect of managing UK upland grasslands on the stock and fractional distribution of SOC

The aim of Chapter 3 was to determine the effects of conventional pasture and traditional hay meadow management regimes on the stock and fractional distribution of SOC in selected UK upland grasslands (thesis objective 2). Results showed that the upland grasslands stored large amount of organic C (59 – 101 Mg ha^{-1}) in the top 15 cm of their soils. The conventional pasture site receiving inorganic N addition and cut for silage had a significantly higher SOC stock (101 Mg ha^{-1}) than all other sites (under both conventional pasture without inorganic N addition and hay meadow) with SOC stock ranging from 59 to 69 Mg ha^{-1}. There was no significant influence of management regime on the fractional distribution of the SOC stock. Across all the grasslands studied, regardless of management, about 60% of the SOC stock was physically protected in a relatively stable form within soil aggregates and on the surfaces of clay and silt particles. This indicates that a greater proportion of the SOC stock in the grasslands is less vulnerable to loss, thus the grasslands have a great potential to contribute to climate change mitigation. The labile SOC pool of the conventional pasture was not significantly different from that of the hay meadow; which indicates that at least some of the SOC gained under inorganic N addition were transferred to a potentially stable form.

The physically protected SOC and the labile SOC reported in this thesis are within the range reported in other published upland grassland studies (Figure 6.1). However, the labile SOC pool reported in this thesis (1.5-2.2%) is much smaller than in most other published studies (1.5-18.0%; Figure 6.1). This could be due to the low recovery of C during the fractionation process as discussed in Section 3.4.3 or differences in management such as the type of livestock used for grazing. For example, Leifeld and Fuhrer (2009) reported about 50% higher labile SOC in conventional pasture sites grazed by cattle than in their hay meadow counterparts grazed by sheep in Switzerland. In contrast, the UK upland grasslands reported in this thesis were all moderately grazed by sheep and there was no significant difference in labile SOC between the conventional pasture and hay meadow sites.

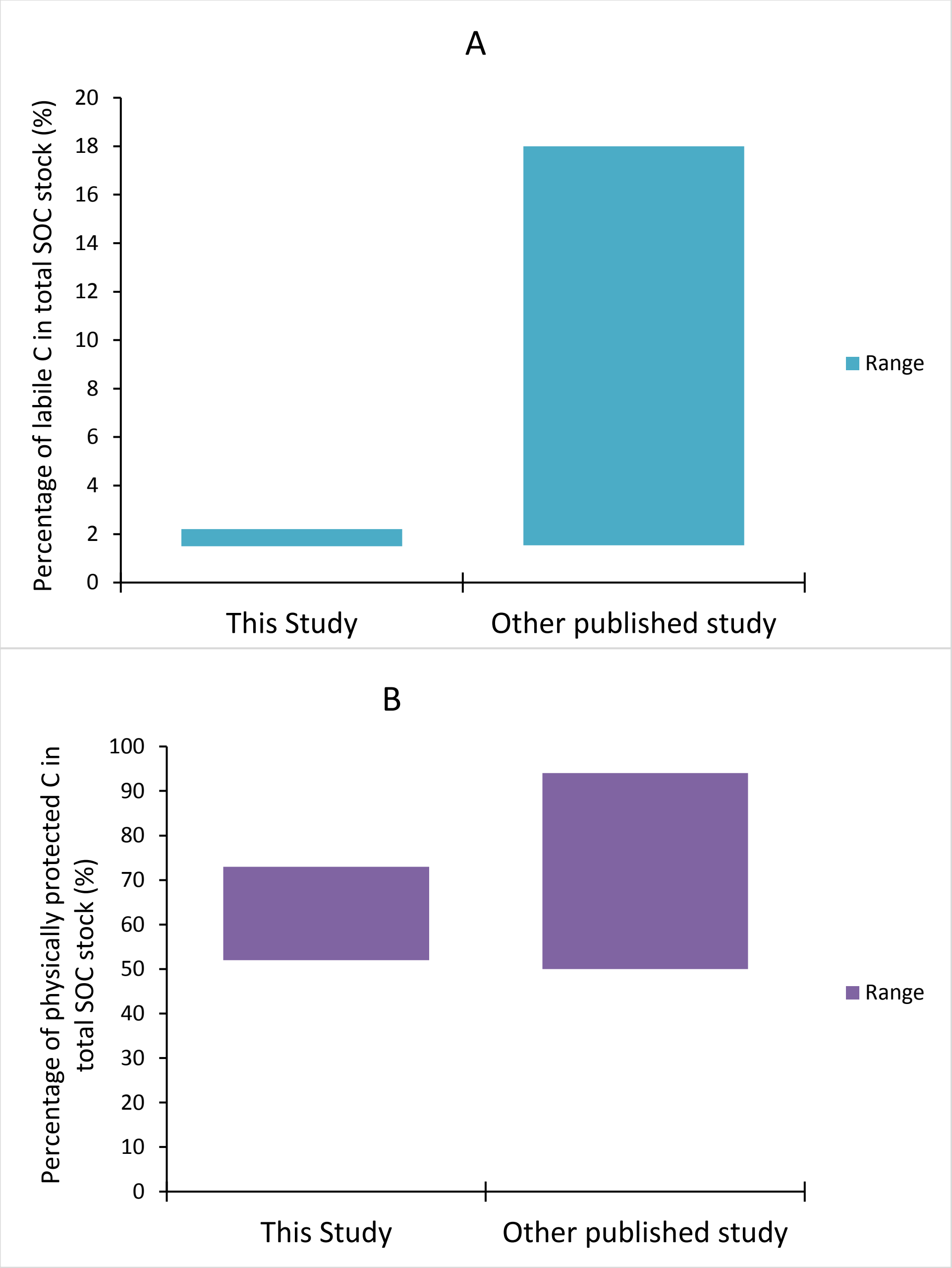

Figure 6.1: A comparison of (A) labile SOC and (B) physically protected SOC in this thesis with other published upland grassland studies (n = 13) reviewed in the thesis. SOC = soil organic carbon.

6.1.3 The effect of managing UK upland grasslands on the ecosystem CO_2 and CH_4 fluxes

Chapter 4 sought to assess the response of ecosystem CO_2 and CH_4 fluxes to conventional pasture and hay meadow management regimes in the UK upland grasslands (thesis objective 3). Results showed that CH_4 flux was generally very small (-0.36 to -0.44 g CH_4 m^{-2} $year^{-1}$) in all the grasslands and the fluxes of C were dominated by CO_2. The upland grasslands were a net atmospheric C sink (1822 – 2758 g CO_2-eq m^{-2} $year^{-1}$), with the conventional pasture site that receives the highest N inputs having the greatest ecosystem C uptake (2758 g CO_2-eq m^{-2} $year^{-1}$). There was no significant difference between the NEE of the conventional pasture and hay meadow fields in Nidderdale, whereas the conventional pasture in Ribblesdale had a significantly more negative NEE (C uptake) than its hay meadow counterpart. The implication here is that the hay meadows were generally less efficient C sinks than the conventionally managed pastures.

6.1.4 Potential impacts of climate change on the biomass productivity and C fluxes of managed UK upland grasslands

The aim of Chapter 5 was to assess the effects of experimental climate change on plant biomass production, MBC, NEE, and DOC flux in UK upland grasslands (thesis objective 4). N-rich mesocosms extracted from the conventional pasture grasslands and N-poor mesocosms extracted from the hay meadow grasslands were used for the experiment. Results showed that experimental summer climate of late 21st Century (+3°C increase in temperature and -23% decrease in precipitation) resulted in a significant decline in the net ecosystem C sink (-13 to -29%) and plant biomass productivity (-29 to -77%) of the mesocosms, regardless of their N status. Supplemental precipitation could not reverse the negative effects of warming on the net ecosystem C uptake and plant biomass production. The DOC flux was not significantly affected by the experimental climate change. The implication of the findings here is that the management regimes in the upland grasslands may not influence the impacts of climate change on grassland productivity and C fluxes.

6.2 Implications of research findings

The UK upland grasslands reported in this thesis have high SOC stock that is predominantly protected in a relatively stable form. The grasslands also have significant potential for sequestering atmospheric C, just like other temperate grasslands (Jones and Donelly, 2004). Interestingly, in this study the C sink in the upland grasslands within the one year of monitoring was greater than the values reported in other European grasslands (Figure 6.2) and temperate grasslands outside Europe (Gilmanov *et al.,* 2010). At the current rate of C uptake, these grasslands have a great potential for contributing to climate change mitigation, provided the C sink trend is sustained before the ecosystem reaches its C saturation point. Although the removal of C in silage/hay may extend the time for C saturation.

The meta-analysis results in this study showed that within the temperate zone, management-induced SOC loss correlated negatively with increasing MAP and MAT, which suggests that future warming in the temperate grasslands will enhance SOC stock. However, experimental summer warming and drought lead to a significant decline in the net uptake of atmospheric C by the UK upland grasslands, regardless of management regimes. This indicates that future climate change has the potential to turn the UK upland grasslands that are currently C sinks into C sources.

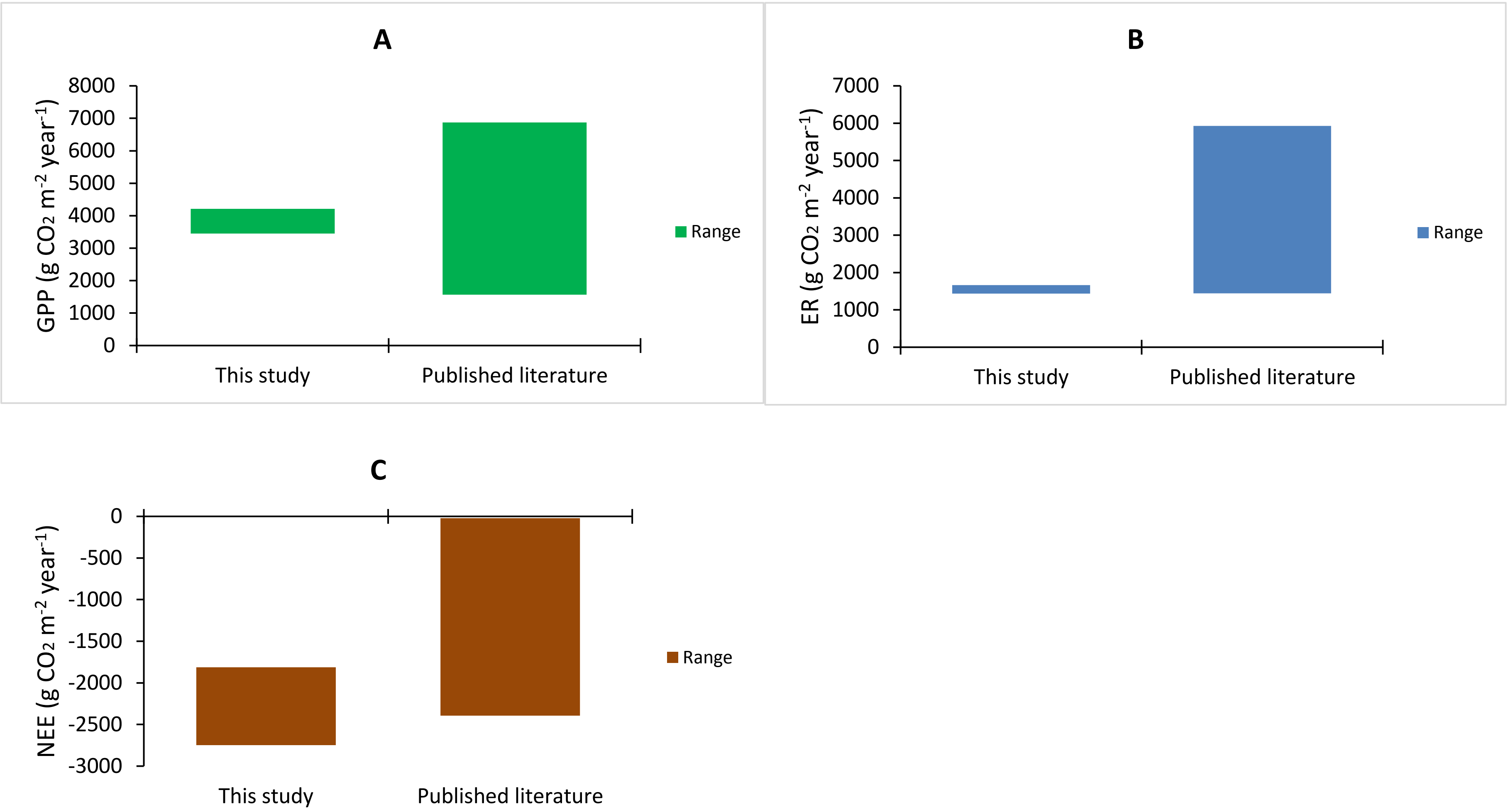

Figure 6.2: A comparison of (A) gross primary productivity-GPP, (B) ecosystem respiration-ER, and (C) net ecosystem exchange-NEE in this thesis with other published grassland studies (Table A4.1) in Europe.

6.2.1 Management of the UK upland grasslands: implications for developing new agri-environment schemes for multiple benefits

The UK upland grasslands are currently managed typically as conventional pasture or traditional hay meadow under agri-environment schemes. The targeted outcomes of these management regimes are different. The conventional pasture management regime, which usually involves the application of inorganic and organic fertilizers, aims at increasing forage productivity for livestock production. In contrast, the traditional hay meadow under agri-environment schemes receives no inorganic fertilizers and is concerned with the restoration, protection and enhancement of biodiversity (Critchley *et al.,* 2004). The agri-environment schemes in England are also designed to protect soils from erosion and contribute to climate change mitigation (Natural England, 2009). Although the major goal of agri-environment schemes is the same across upland grasslands, specific management activities at each site depend on site condition, especially soil and vegetation. For example, the two locations that I studied are under similar agri-environment schemes but had slightly different management practices; the site with acid-forming parent materials were limed whereas the site on limestone parent material received no lime.

Agricultural lands have great potential for sequestering atmospheric C (Minasny *et al.,* 2017) but evidence is still lacking on the influence of different management practices across soil-land use-ecoregion combinations. Hence, it is worth investigating whether existing agri-environment schemes in specific environments can deliver C sequestration in addition to primary objectives. Improved understanding of the impacts of the existing management regimes will also ensure that the best elements of these extant schemes are incorporated into new agri-environment management policies.

This study showed that the grasslands under both conventional pasture and hay meadow management regimes were net C sinks. However, the conventional pasture that receives inorganic N fertilizers (50 kg N/ha/year) and with greater soil available nitrate, had the greatest C uptake (-2758 g CO_2-eq m^{-2} $year^{-1}$) and SOC stock (101 Mg ha^{-1}) compared to other fields with no addition of inorganic fertilizer. This is consistent with the results found in other managed grasslands as shown in Chapter 2, where inorganic N fertilizers led to a significant increase (+8.1%) in SOC stock. This is not surprising because N addition is known to stimulate vegetation growth and the return of biomass litter to the soil, which subsequently enhances soil C storage (Fornara *et al.,* 2013; Song *et al.,* 2014; Yue *et al.,* 2016). Agri-environment schemes discourage the addition of N fertilizers to encourage biodiversity, and this has caused significant reductions in grassland productivity. For example, Taylor and Morecroft (2009) reported a 50% reduction in grassland productivity after N fertilization ceased due to the introduction of an agri-environment scheme in some grasslands in England. This reported negative effect of agri-environment management scheme on biomass production makes one wonder if the scheme could be of any benefit to climate change mitigation. In the long-term, the scheme may have some added benefits from e.g. reduced erosion due to lower grazing pressure. However, whether this added benefit is actually derived from agri-environment schemes needs to be investigated in future studies. Even if the benefit is derived, future studies should investigate the implication for C sequestration.

I found non-significant differences in CO_2 and CH_4 emissions between the hay meadow fields under agri-environment schemes and the conventional pasture fields (Chapter 4). Also, the grasslands under agri-environment schemes had similar or lower SOC stock than their conventional pasture counterparts (Chapter 3). This suggests that the existing agri-environment scheme may not provide C sequestration benefits in the UK upland grasslands, and may contribute little or nothing to climate change mitigation. The effectiveness of the agri-environment scheme in achieving its main goal of promoting biodiversity has also been questioned (Kleijn and Sutherland, 2003), and the best the scheme could provide is marginal to moderate biodiversity benefits (Batáry *et al.,* 2015; Kleijn *et al.,* 2006). A recent study in Austria (Hülber *et al.,* 2017) has reported that there were significant losses in the species richness of grasslands despite 20 years of agri-environment schemes. All these findings suggest that the current agri-environment schemes have not lived up to the benefits expected of them in terms of increased biodiversity and climate change mitigation. This does not mean that agri-environment schemes should be stopped completely in the UK uplands but it calls for a consideration of new schemes capable of delivering multiple benefits of increasing forage productivity, enhancing SOC stock and promoting biodiversity. This will ensure that the management of the uplands is in line with the Biodiversity 2020 strategy in England (DEFRA, 2011) which targets an increase in food production alongside the provision of other ecosystem services.

To achieve multiple ecosystem services, prescriptions of any new agri-environment scheme need to be based on site-specific characteristics (Critchley *et al.,* 2004), taking into account the existing specific management activities such as liming. For example, liming removes limitations imposed by soil acidity (Newbould, 1985), which subsequently enhances vegetation growth (Kirkham *et al.,* 2014) and may lead to an increase in SOC stock. As shown in Chapter 3, the SOC stock in the limed Nidderdale soils (59 – 101 Mg ha^{-1}) encompassed the range of SOC stock in the Ribblesdale soils (65 – 69 Mg ha^{-1}) and other reported UK grasslands (e.g. 85 Mg ha^{-1}; Carey *et al.,* 2008). Also, Chapter 2 revealed that liming resulted in a 5.8% increase in the SOC stock of global grasslands. This therefore makes liming a promising strategy for increasing forage production in the acidic upland grasslands, and an effective management strategy that will contribute to mitigating global warming (Fornara *et al.,* 2011). Hence, liming should be retained and be an integral part of new agri-environment schemes in acidic upland grasslands in the UK. However, it is necessary that lime is applied when the soil pH is below the threshold pH (6.0) above which liming is not required in grasslands in England and Wales (DEFRA, 2010). Adding lime only when the soil pH falls below recommended threshold level will ensure that liming does not increase the mineralization of N, which could get leached as nitrate into water bodies thereby reducing water quality (Holland *et al.,* 2018).

Grazing is an important factor that influences the effects of agri-environment schemes on grasslands (Critchley *et al.,* 2004). This study showed that heavy grazing resulted in a significant reduction (-27%) in the stock of SOC in global grasslands (Chapter 2). However, no significant differences in SOC between the UK upland grasslands (Chapter 3) could be attributed to grazing. The high C stock in all the UK upland grasslands studied suggests that the current grazing regime may not lead to SOC loss. Grazing-induced net SOC loss is mostly caused by excessive removal of vegetation which consequently leads to limited litter returns to the soil (Han *et al.,* 2008). Based on the classification of grazing intensity in England and Wales (DEFRA, 2010), all the sites I studied were moderately grazed. Moderate grazing tends to enhance SOC storage due to the regrowth of vegetation with greater biomass than initially removed by the grazing livestock (Tanentzap and Coomes, 2012). This subsequently increases C inputs to the soil. The current grazing regimes can therefore be maintained even under new agri-environment schemes aimed at increasing forage productivity and SOC stock. However, whether the current stocking rate is maintained or reduced will depend on the level of stress imposed by future climate change on grassland productivity.

Considering the direct positive relationship between available soil nitrate and GPP in the upland grasslands (Chapter 4) and that SOC stock was greatest in sites receiving modest N addition (Chapter 3), it may be necessary to include modest additions of inorganic N (at most 50 kg N ha^{-1}) in any new agri-environment scheme rather than nil. I also encourage the addition of organic manure because it has been reported to significantly increase soil C storage more than inorganic fertilizers (Jones *et al.*, 2006). The Nidderdale site with the highest SOC stock receives 6 t ha^{-1} year^{-1} of FYM, and this rate of FYM has been reported to improve vegetation quality and species richness (Kirkham *et al.*, 2014). The addition of N as a mixture of mineral fertilizers and organic manure may be a viable management option that will enhance forage productivity, promote biodiversity, and maintain or possibly enhance soil C stock in the upland grasslands. I am not aware of any study that has investigated the effects of mixed organic and inorganic N fertilizer application on the productivity and C sequestration of the UK upland grasslands. However, the environmental and economic impacts of agri-environment schemes in the French West Indies that incorporated the addition of organic and moderate levels of inorganic fertilizers has been investigated using a modelling approach (Blazy *et al.*, 2015). The result of the modelling study showed that relative to the conventional management involving the addition of high levels of inorganic fertilizers, the option of adding a mixture of compost and moderate levels of inorganic N fertilizers increased C sequestration by 350%, reduced nitrate leaching by 45% and maintained vegetation quality after five years of implementation (Blazy *et al.*, 2015). The sites studied by Blazy *et al.* (2015) differ from the UK upland grasslands in both climate and soil type, hence the UK upland grasslands may respond differently to the N fertilizer regime in the French West Indies. There is therefore need to investigate this in future studies.

Overall, the liming and grazing regimes in the UK upland grasslands can be maintained without reducing the sequestration of C by these ecosystems. However, to enhance forage productivity and possibly C storage, future agri-environment schemes could consider incorporating the addition of moderate levels of inorganic N fertilizers and organic manure in the suite of management prescriptions.

6.2.2 Managing the UK upland grasslands under future climate: implications for soil carbon sequestration and biomass production

Previous studies (e.g. Chapman *et al.*, 2009; Emmet *et al.*, 2010; Smith *et al.*, 2007) have shown that climate change in the 20th Century (see Section 1.3) did not cause any significant change in SOC stock in the UK. Some recorded reductions in the UK's SOC stock in the 20th Century (Bellamy *et al.,* 2005) were mostly attributed to changes in management practices (Barraclough *et al.,* 2015; Kirk and Bellamy, 2010; Smith *et al.,* 2007) rather than climate change. Under current climatic conditions and management regimes, the UK upland grasslands reported in this study were a net atmospheric C sink (Chapter 4). Also, the greatest proportion (60%) of the SOC stock in the grasslands was physically protected in stable soil fractions, within aggregates and on the surfaces of silt and clay particles (Chapter 3). The protection of organic C in stable fractions has been shown to increase its residence time in the soil (Leifeld and Fuhrer, 2009) because decomposition processes are restricted (Conant *et al.,* 2011). The results of this thesis therefore indicate that the SOC stock in the uplands are maintained under current management regimes. However, an important issue of concern is how C dynamics in the upland grasslands will change under future climate.

The results in Chapter 5 suggest that climate change in the 21st Century is most likely going to cause a significant reduction in the C sink capacity of the UK upland grasslands. Based on this study, it is likely also that future climate change will reduce biomass productivity which could subsequently affect livestock production. These climate change impacts were observed regardless of whether the grasslands were under conventional pasture or hay meadow management regimes. This suggests that future climate change is likely going to trump the potential beneficial effects of the current management regimes on the maintenance of C sequestration in the grasslands.

Some modifications of current management regimes may therefore be necessary in order to protect upland grassland productivity and C sequestration. As recommended in Section 6.2.1, carefully controlled additions of N as both inorganic fertilizers and organic manure should be included in the prescriptions of new agri-environment schemes. This may help sustain the productivity of the grasslands in the long term under climate change, provided soil moisture is not limited. However, the negative effects of experimental climate change observed in this study was attributed to soil moisture limitations (Chapter 5). Hence, soil water management is an area that need to be given serious consideration in managing the uplands. A water management option that can be easily incorporated into land management prescriptions, without imposing economic burden on the farmers and land managers, is hay mulching.

Hay mulching refers to the cutting of the aboveground vegetation into small pieces and spreading it on the soil surface (Gaisler *et al*., 2013). It conserves soil moisture (Xing *et al.*, 2012) and reduces soil temperature (Dudás *et al.*, 2016). Hay mulching is also a low-cost management practice which has been shown to deliver multiple ecosystem services of increasing soil nutrient status, enhancing species richness and diversity, increasing biomass productivity and soil C stock. For example, a 13-year experiment in a mountain meadow in Czech Republic showed that hay mulching (relative to fallowing or cutting and removal of vegetation) promoted plant species richness and functional diversity as well as high biomass production (Doležal *et al.*, 2011). Higher resilience of grasslands to extreme climatic conditions has also been attributed to hay mulching (Hamre *et al.*, 2010).

With the numerous benefits associated with hay mulching, it appears to be a viable management option that will help to achieve the overall aim of agri-environment schemes (i.e. to promote biodiversity) and at the same time, mitigate negative effects of climate change on the upland grasslands. Under the existing management regime, the upland grasslands are grazed by lambing sheep in spring, the livestock is taken out of the field in mid-May for the vegetation to regrow, and the vegetation is cut for hay in July. Hay mulching can be included in this cycle by spreading some of the vegetation cut during hay-making on the field surface in summer. The downside to hay mulching is that the farmer may not have sufficient biomass to save as hay or silage to feed animals over winter. However, hay mulching is still an option that land managers may need to consider in the future.

The new post-BREXIT agricultural policy proposed by the Department for Environment, Food and Rural Affairs (DEFRA) UK, aims to give monetary incentives to farmers and land managers who provide public goods such as increasing biodiversity and C sequestration to mitigate climate change (DEFRA, 2018). In the proposal, it was stated that possible options will be explored on how to best support the uplands, where farming activities are more restricted than in the lowlands due to challenging landscape characteristics such as low soil fertility and steep slopes. The immediate focus of the new scheme will be on improving productivity as well as profitability of farm businesses which are currently dependent on subsidies (DEFRA, 2018). Controlled N addition as both inorganic fertilizers and organic manure is an option that could be included in future agri-environment schemes to enhance grassland productivity and C sequestration. Also, hay mulching is a good option that can provide the targeted public goods sought by the proposed agri-environment policy, in addition to enhancing forage productivity for sustaining livestock production. It is an easy and more profitable option for farmers as there are little or no economic costs associated with the adoption. Also, hay mulching does not require any major changes in the existing management practices.

6.3 Limitations of the study

This thesis provided new results that improved understanding of the dynamics of C in UK upland grasslands underlain by mineral/organo-mineral soils and under two typical management regimes and experimental climate change. However, there are a few limitations of this work that need to be explored further in order to make more reliable future management decisions.

The focus of this thesis was mostly on the net uptake of atmospheric C by the grasslands and the stock of SOC, whereas the net ecosystem C balance (NECB) was not investigated. The NECB accounts for all possible ecosystem C imports (e.g. supplementary livestock feed and organic manure) and exports (e.g. harvested plant biomass, C offtake in meat and milk, leaching and erosion C losses) (Mudge *et al.*, 2011). The NECB is more comprehensive than NEE and is a better measure of C sink/source of an ecosystem. Despite the importance of accounting for the overall C balance of a managed ecosystem, a search through the published studies I reviewed in this thesis showed that the NECB is not often considered in C flux studies in managed grasslands (see Figure 6.3). For example, only five studies (Ammann *et al.*, 2007; Chang *et al.*, 2015; Elsgaard *et al.*, 2012; Jones *et al.*, 2017; Soussana *et al.*, 2007) in my reviewed articles investigated the NECB in managed grasslands. Four out of these five studies did not account for C losses via leaching and erosion, and none of the studies estimated C export in milk and meat. Some of the studies (e.g. Ammann *et al.*, 2007; Chang *et al.*, 2015) had to rely on modelling in order to estimate some components of the C cycle. This shows that NECB is a common limitation in many C flux studies and is a gap in C cycle understanding that is challenging to address because: 1) researchers have to rely on good record-keeping by farmers for information on biomass harvest, meat and milk offtake; 2) internal cycling via animal waste is hard to quantify; and 3) monitoring C loss via erosion and leaching is time-consuming. Despite these challenges, assessing NECB is necessary because without it, determining if soils are sequestering C or not will be hard. Lack of NECB data of managed ecosystems will make it difficult to assess the performance of C sequestration policies and initiatives such as the “4 per mille” initiative discussed in Section 1.

The number of grasslands used for this study was relatively small due to limited resources available for the research and the difficulty in finding appropriate sites. Two locations in northern England that are 20 km apart, with each location having two contrasting management regimes were reported in this thesis. Replicate plots had to be established within each field because multiple fields with similar management regimes and soil types could not be found at each location. This pseudo-replication approach has the tendency not to properly incorporate the true nature of randomness in the collected data (Millar and Anderson, 2004). However, not having multiple fields with similar management regimes at a location is perhaps a common challenge of working in upland agricultural environments. Due to the inherent limiting characteristics of the uplands such as poor soil fertility and steep topography, farmers may have different parts of their land under different agri-environment management schemes. Also, the management regime may change over a relatively short period of time e.g. a few years, if the farmer decides to switch agri-environment schemes or opt out of the schemes completely.

The application of N fertilizers, which is common practice in the conventional pastures, has the tendency to increase the emission of N_2O into the atmosphere (Jarvis *et al.*, 2001), and this was not investigated in this thesis. Hence, the results in this thesis are not sufficient to give a full account of the GHG balance in the grasslands.

The experiments/observational studies in this thesis were relatively short term (≤ 12 months). As a result, the changes in the stock of SOC of the UK upland grasslands over 6-10 years as a consequence of management (Smith, 2004) or over a longer period due to climate change could not be assessed. Inter-annual and inter-decadal variability in C fluxes such as NEE could not also be accounted for in this thesis because it required continuous C flux measurements lasting for at least a decade (Luo *et al.*, 2012). Assessing the inter-annual variability in the C fluxes of grassland ecosystems is important as it helps to understand the adaptive changes in plant and microbial species’ physiology and community composition overtime (Grime *et al.*, 2000). Thus, there is a danger of overestimating the C sink/source capacity of the UK upland grasslands when their inter-annual and inter-decadal C flux variability is not known.

The climate manipulation experiment in this thesis (Chapter 5) was conducted in an environmental room and with small soil mesocosms. Although environmental room experiments try to simulate field conditions, the factors operating in the field are complex and may not be fully represented. For example, CO_2 might have been lost from the open bottom of the soil mesocosms. Losing CO_2 from the mesocosms increases the chances of underestimating ER, which makes it nearly impossible to measure the true ecosystem C balance. The excessive soil moisture loss found in Chapter 5 could also be due to the open-ended nature of the soil cores used. The result could be different in field conditions considering the large soil volume and denser sub-soil layers which help to retain water for plant use in the field. Hence, it is possible for field conditions to offer greater resilience to climate change than observed under experimental conditions.

The ER values reported in Chapter 4 are lower than ER values reported for other temperate grasslands (e.g. Table A4.1). Although the relatively cooler and wetter conditions at my sites may explain the lower ER compared to the temperate grasslands reported in Table A4.1 (see Section 4.4), the ER might have been underestimated due to: 1) the relatively long duration (25 minutes) of field measurement using closed chambers; and 2) the use of aggregate climate data for modelling. Compared to the NEE that was measured every 20 seconds for a period of 120 seconds with an infrared gas analyser, samples for ER were collected every 5 minutes for a period of 25 minutes and were subsequently analysed with a GC (Section 4.2.3). Enclosing vegetation within my chambers for up to 25 minutes during ER measurement might have greatly reduced the rate of CO_2 diffusion thereby leading to low ER. Similarly, the use of mean daily soil temperature data rather than half-hourly or hourly data for gap-filling might have led to an underestimation of ER. This is likely because of the difference in distribution between the non-linear function (Equation 4.6) used for modelling ER and the linearly weighted daily soil temperature data used for gap-filling. Accounting for these factors in C flux measurements helps to reduce bias and to minimize overestimation of C sink in grasslands.

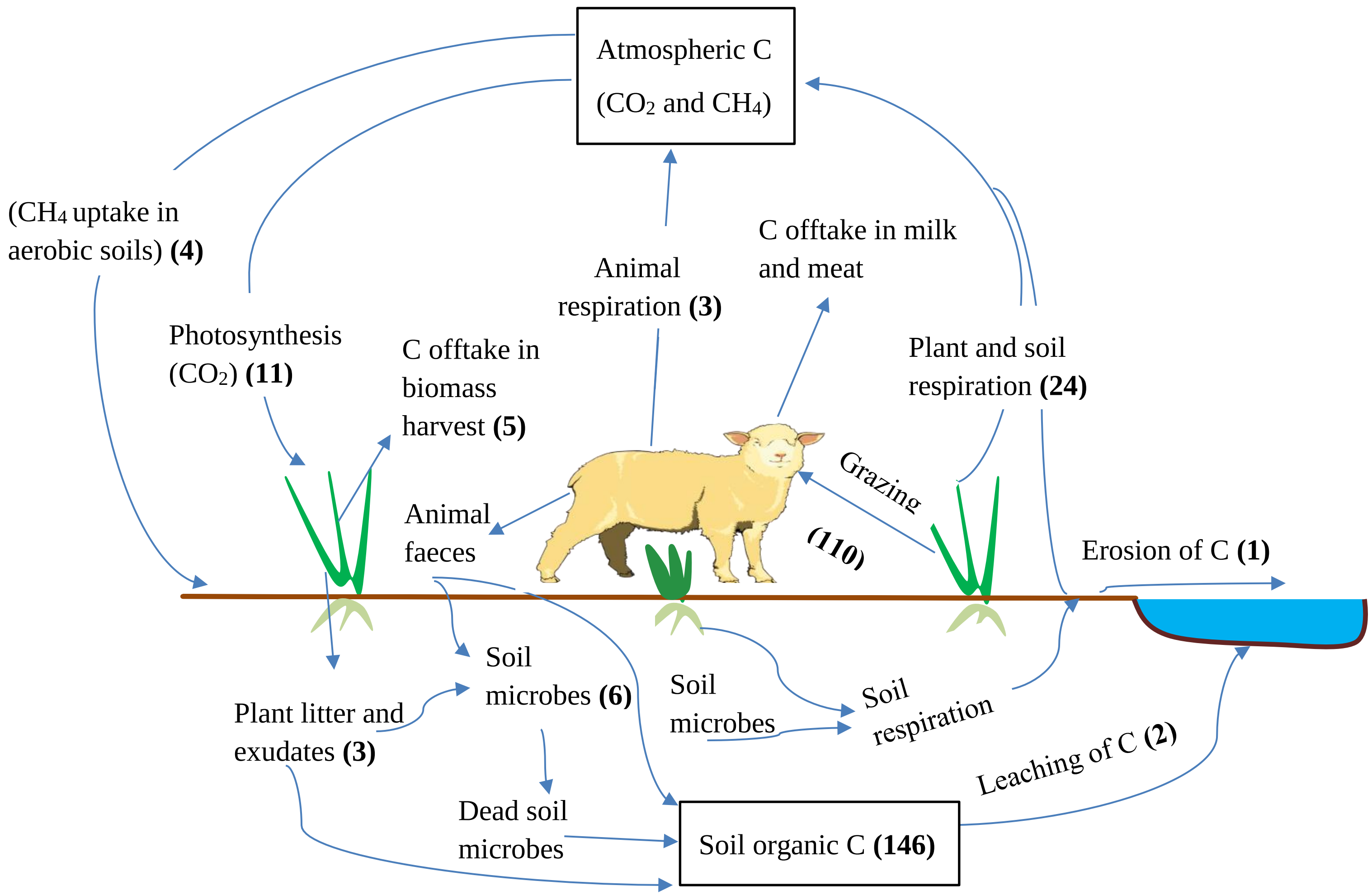

Figure 6.3: Pathways of carbon (C) cycling in grasslands and the number of grassland studies globally (in bold font) reviewed in this thesis that investigated them.

6.4 Direction for further research

In order to formulate evidence-based management policies that are reliable and sustainable in the UK upland grasslands, there is need for future research to focus on the limitations and gaps identified in the preceding sections. However, it is necessary to prioritise research needs and make efficient use of research funds to design a new agricultural land management scheme for the UK, given that policy makers need to make decisions soon on post-BREXIT land management policies. In DEFRA's plan for the implementation of a new agri-environment management scheme in England, there will be a transition period between the end of the existing scheme (perhaps in March 2019) and the beginning of a new regime few years later (DEFRA, 2018). It was stated in DEFRA's consultation document that by gradually withdrawing direct payments from the existing scheme, funds will be made available for pilot studies on proposed new management options in preparation for their full introduction. This means that there is urgent need for areas of research focus to be clearly identified and targeted in pilot studies.

As discussed in the preceding sections, there are options for low-risk management changes that should be explored as a priority in the uplands: 1) incorporation of hay mulching into the prescriptions of the current agri-environment management schemes; and 2) adding moderate levels of inorganic fertilizers alongside organic manures. Some key questions that need to be answered are: 1) how will the uplands respond to hay mulching? 2) Will hay mulching substitute for organic manure given that it eventually becomes SOM? 3) What will be the NECB of the grasslands? 4) What will be the overall GHG balance of the grasslands if inorganic N fertilizers are added?

To address these questions, there is need for a bigger research project that will: 1) identify more upland grasslands that are managed as traditional hay meadows under agri-environment schemes or conventional pastures; 2) select pilot plots where hay mulching and a mixture of inorganic and organic manures will be applied separately and in combination; 3) monitor the NECB and GHG balance of the grasslands over a relatively longer period than in this thesis, at least within the few years of transition proposed by DEFRA. This will provide information that can be reliably used across UK upland grasslands for management policy decisions.

Assessing the NECB and a full accounting of the GHG balance of the grasslands, which entails the monitoring of CH_4, CO_2 and N_2O fluxes (Soussana *et al.*, 2007; Chang *et al.*,

2015) will help to fully understand the implications of the various upland grassland management regimes for climate change mitigation. Finally, future climate manipulative experiments should be carried out under field conditions, rather than in controlled laboratory condition, so as to obtain results that reflect the complex interactions in the field that are more realistic.

6.5 Conclusion

The UK upland grasslands investigated in this thesis were a net atmospheric C sink, with a significant SOC stock which was predominantly protected in the soil mineral mass. The C uptake in these grasslands was greater in the conventional pasture fields receiving the highest N addition, and where the soil available nitrate was greatest. The traditional hay meadow under agri-environment scheme has lower C sequestration benefits than the conventional pastures. Experimental warming and altered precipitation resulted in a significant reduction in plant biomass production and C uptake in mesocosms extracted from both the conventional pasture and hay meadow fields. This indicates that warmer climate and drought by the end of the 21st Century will most likely lead to a significant decline in both C sink and forage production in the UK upland grasslands. Moderate levels of inorganic N fertilizers alongside organic manures is recommended to enhance biomass production and C sequestration in the traditional hay meadows. Similarly, hay mulching is recommended as a viable management option to mitigate against the potential negative effects of climate change on both biomass productivity and C sequestration. Pilot studies on a greater number of managed grasslands are needed to test the effectiveness of the recommended management changes for providing targeted outcomes. Specific areas that require further research are: 1) NECB, 2) overall GHG balance, and 3) field climate manipulation experiments. An improved understanding of these areas will increase the evidence base to support new agricultural land management policies in the UK uplands that will ensure sustainable livestock production and climate change mitigation.

6.6 References

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Appendices

Appendix 2.1: Studies used for meta-analysis.

Table A2.1**:** Site characteristics of studies selected for meta-analysis and average effect size of management on SOC stock.

Country	Latitude (°)	Longitude (°)	Altitude (m)	MAT (°C)	MAP (mm)	Soil type	Management type	Nature of grassland	Nos	Effect size (%)	Author(s)
Spain	42.98	-2.1	876 - 1224	12.4	1400	Calcic Luvisol	Grazing exclusion	Permanent grassland	2	-0.4	Aldezabal et al 2015
Uruguay	-31.9	-58.25	14	17.4	1099	Mollisol	Grazing exclusion	Permanent grassland	1	-0.28	Altesor et al 2006
China	37.95	107	1430	7.5	280	Light sierozem	Grazing exclusion	Permanent grassland	1	-16.0	An and Li 2015
Argentina	-38.75	-63.75	94	15	400	Petrocalcic Calciustolls	Grazing exclusion	Permanent grassland	1	3.4	Andrioli et al 2010
Australia	-37.79	145.88	87	14.8	666	Sodosol	Liming	Permanent grassland	5	-4.2	Aye et al 2016
India	32	78	4325	-4.3	495	Sandy loam	Grazing exclusion	Permanent grassland	1	6.6	Bagchi and Ritchie 2010
China	45.74	115.43	1105	-0.26 – 1.3	176 – 353	Chestnut soil	Continuous grazing	Permanent grassland	3	-11.2	Bai et al 2012
New Zealand	-43.18 - -43.45	171.56	810 – 870	8.2 – 8.9	1000	Typic Orthic Brown	Grazing exclusion	Permanent grassland	2	6.5	Basher and Lynn 1996

United Kingdom	53.23	-4.02	30	11	1250	nr	Grazing exclusion	Improved pasture	1	2.6	Boddy et al 2007
China	41.78	111.88	1450	3.4	280	Light chestnut soil	Grazing exclusion	Permanent grassland	3	-4.8	Cao et al 2013
Brazil	-18.97	-56.62	106	24	1125	nr	Continuous grazing	Cultivated pasture/Permanent grassland	1	-36.85	Cardoso et al 2010
China	43.63	116.7	1255	2.4	279	Calcic Chernozem	Grazing exclusion	Permanent grassland	1	-29.1	Chen and Tang 2016
China	43.93	116.37	1400	0.6	350	Chestnut soil	Grazing exclusion	Permanent grassland	1	-7.4	Chen et al 2008
China	42.92	120.7	385	6.8	366	Orthi-Sandic	Grazing exclusion	Permanent grassland	1	-41.6	Chen et al 2012
China	36.23	116.4	1950	7.0	425	Calci-Orthic Aridisol	Grazing exclusion	Permanent grassland	1	4.7	Cheng et al 2016
China	43.53	116.67	1274	0.2	350	Kastanozems	Grazing exclusion	Permanent grassland	3	-2.9	Cui et al 2005
New Zealand	-43.78	171.8	160	11	740	Udic Ustrochrept	Fertilizer (P)	Cultivated/Sown pasture	2	-1.3	Curtin et al 2007
Brazil	-28.93	-54.33	425	19	1850	Rhodic Haplorthox	Continuous grazing	Sown pasture	4	-22.9	da Silva et al 2014
France	45.72	3.02	880	8.7	780	Cambisol	Continuous grazing	Permanent grassland	2	-6.9	Damien et al 2015
China	36.87	106.33	1924	6.7	410	Aeolian soil	Grazing exclusion	Permanent grassland	1	-20	Deng et al 2014

USA	38.87 – 40.82	-96.58 - -107.77	377 - 2151	8.6 – 12.8	321 – 835	Mollisol; Aridisol	Grazing exclusion	Permanent grassland	3	6.8	Derner et al 2006
China	33.62	99.78	4000	-1.3	590	nr	Continuous grazing	Permanent grassland	6	-11.4	Dong et al 2012
Canada	50.75	-120.42	725	8.39	270	Chernozems	Grazing exclusion	Permanent grassland	1	-7.3	Evans et al 2012
China	32.08	93.75	4340	-1.1	548	nr	Grazing exclusion	Permanent grassland	1	-19.5	Fan et al 2013
China	37.62	101.32	3220	-1.7	643	Cambisol	Fertilizer (N)	Permanent grassland	9	-2.2	Fang et al 2014
USA	45.4	-93.2	277	6.7	801	Fine sand	Fertilizer (N)	Successional grassland	7	29.5	Fornara and Tilman 2012
United Kingdom	51.41	-0.64	63	9.6	754	Sand	Fertilizer (N and P)	Semi-natural grassland	2	19.7	Fornara et al 2013
USA	35.42	-99.08	490	15	766	Cordell	Continuous grazing	Permanent grassland	2	-25.5	Fuhlendorf et al 2002
Brazil	-22.3	-48.3	595	22	1230	Latosol	Continuous grazing	Sown grassland	2	-8.2	Garcia and Nahas 2007
USA	39.28	-111.5	2735 – 3170	1.3	932	Argic Cryoborolls	Grazing exclusion	Permanent grassland	10	-6.2	Gill 2007
Argentina	-41.68	-70.27	1355	4.5	150	Petrocalcic Calciorthids	Grazing exclusion	Permanent grassland	2	-6.5	Golluscio et al 2009
China	42.89	83.71	2500	-4.8	266	nr	Fertilizer (N)	Permanent grassland	4	-1.0	Gong et al 2014

Iceland	65.04	-14.95	60	4.1	501	Gleyic andosol	Fertilizer (N)	Permanent grassland	4	9.8	Gudmundsson et al 2004
China	44.03 – 43.27	116.15 - 116.38	1090 – 1260	1.7	350	Kastanozems	Grazing exclusion	Permanent grassland	2	-13.5	Guo et al 2016
China	35.53	99.85	3440	1.7	582	Haplic Kastanozems	Grazing exclusion	Permanent grassland	1	27.3	Hafner et al 2012
Argentina	-38.75	-63.75	94	15	400	Calciustolls	Grazing exclusion	Permanent grassland/isolated woody plants	1	-2.2	Harris et al 2007
China	43.51	116.65	1200	1.1	345	Calcic Kastanozems	Grazing exclusion	Permanent grassland	3	-33.3	He et al 2008
China	43.63	116.7	1200	1.1	345	Calcic Kastanozems	Grazing exclusion	Permanent grassland	1	-31.2	He et al 2009; Wu et al 2008
China	43.55	116.67	1200	1.0	334	Calcic Kastanozems	Continuous grazing	Permanent grassland	6	-7.2	He et al 2011
China	43.55	116.67	1200	0.96	334	Calcic Kastanozems	Grazing exclusion	Permanent grassland	1	-32.93	He et al 2012
China	43.55	116.67	1200	1.1	345	Calcic Chernozem	Fertilizer (N and P)	Permanent grassland	10	7.9	He et al 2013
Canada	49.40 – 52.05	-110.18 – 114.31	790 – 1569	2.3 – 4.4	394 – 441	nr	Grazing exclusion	Permanent grassland	3	-6.2	Hewins et al 2015

Colombia	4.8	-75.4	4000	4.0	929	Cryands	Continuous grazing	Permanent grassland	1	3.48	Hofstede 1995
United Kingdom	51.8 – 55.22	-0.37 - -1.68	87 – 130	5.7 – 6.6	689 – 708	Ochraqualf; Ustrochrept	Fertilizer (N and P)	Permanent grassland	4	0.4	Hopkins et al 2009
China	42.27	112.78	1184	4.9	213	Kastanozem	Continuous grazing	Permanent grassland	1	-7.35	Hou et al 2014
USA	42.73	-89.75	292	7.5	930	Silt loam	Continuous grazing	Sown grassland	2	-7.1	Jackson et al 2015
United Kingdom	55.87	3.2	200	8.3	849	Gleysol	Fertilizer (N)	Semi-natural grassland	2	9.7	Jones et al 2006
United Kingdom	51.83	-0.58	89 – 128	9.6 – 10	649 – 704	Eutric cambisol	Liming	Semi-natural grassland	6	18.1	Kemmitt et al 2006
South Africa	-28.95	26.46	1500	17.8	553	Lixisol	Continuous grazing	Permanent grassland	3	-31.0	Kotze et al 2013
Canada	50.08 – 50.78	-120.41 – -120.43	935 - 1235	4.4	379 - 438	Black Chernozem	Grazing exclusion	Permanent grassland	2	-11.6	Krzic et al 2014
China	4178	111.61	1466	3.8	280	Brown Chermozem	Grazing exclusion	Permanent grassland	3	-8.2	Li et al 2008
China	42.92	120.7	385	6.4	366	Orthi-sandic Entisols	Grazing exclusion	Permanent grassland	1	-59.4	Li et al 2011
Canada	50.78	-113.88	1350	5.5	502	Udic Haplocryoll	Continuous grazing	Permanent grassland	2	-1.0	Li et al 2012a
China	42.97	120.72	360	6.5	366	Cambic Arenosol	Grazing exclusion	Permanent grassland	1	-85.4	Li et al 2012b

China	34.92	102.88	3000	2.4	550	Haplic Calcisols	Fertilizer (N and P)	Permanent grassland	6	-2.7	Li et al 2014a
China	44.25	123.57	153	4.9	364	Meadow solonchaks	Grazing exclusion	Permanent grassland	1	-31.9	Li et al 2014 b
China	31.14	106.84	1650	7.1	359	Cambisol	Continuous grazing	Permanent grassland	3	-2.2	Liu et al 2011
China	41.78 – 43.52	111.88 – 116.68	1200 – 1450	0.7 – 3.4	280 - 335	Kasternozems; Chernozems	Continuous grazing	Permanent grassland	6	1.61	Liu et al 2012
China	43.55	116.7	1250	-0.1	315	Mollisols	Grazing exclusion	Permanent grassland	1	-24.5	Liu et al 2016
Croatia	45.17	15.5	319	15.5	1113	nr	Continuous grazing	Permanent grassland	2	-2.8	Ljubicic et al 2014
China	30.93 – 31.98	84.82 – 91.82	4544 - 4613	-1.4 - -2.5	195 - 394	nr	Grazing exclusion	Permanent grassland	3	31.1	Lu et al 2015a and Lu et al 2015b
China	33.93	102.87	3433	0.9	657	nr	Grazing exclusion	Permanent grassland	1	-39.47	Luan et al 2014
China	32.82	102.57	3485	1.4	749.1	Haplic Kastanozems	Grazing exclusion	Permanent grassland	1	-12.31	Ma et al 2016
Canada	49.28	-121.75	23	10.8	1689	Eutric Eluviated Brunisol	Fertilizer (N)	Sown grassland	2	2.0	Maillard et al 2015
Canada	50.5	-114.05	1113	4.2	450	Typic Boroll	Fertilizer (N)	Permanent grassland	8	34.7	Malhi et al 1997

Canada	50.5	-114.05	1113	4.2	450	Typic Boroll	Fertilizer (N)	Permanent grassland	5	9.5	Malhi et al 1997, Malhi et al 2003
Czech Republic	49.98	12.75	750	6.4	700	Haplic Cambisol	Fertilizer (N)	Sown grassland	3	3.2	Maly et al 2009
Norway	60.71	8.00	1250	-1.5	1000	Leptic podsols	Continuous grazing	Permanent grassland/shrubs	2	1.45	Martinsen et al 2011
New Zealand	-44	170	440	15	550	Typic ustochrepts	Grazing exclusion	Permanent grassland	1	-7.9	McIntosh et al 1997
United Kingdom	54.18	-2.36	490	6.9	1840	nr	Grazing exclusion	Permanent grassland	1	-5.6	Medina-Roldan et al 2012
Ethiopia	13.5	38.5	2584	17.5	609	Aridisols	Grazing exclusion	Permanent grassland/woody vegetation	3	-56.9	Mekuria et al 2011
Spain	43.12	-2.85	950 - 1050	10.1	2000	Dystrudept; Humaquept	Liming	Semi-natural grassland	4	5.8	Mijangos et al 2010
USA	40.05	-105.58	3422	-3.0	900	Inceptisols	Fertilizer (N)	Permanent grassland	1	8.1	Neff et al 2002
Kenya	0	37	1800	20.3	550	Vertisols	Grazing exclusion	Savanna	1	-12.5	Ngatia et al 2015
Thailand	16.43	103.07	181	26.8	1183	Oxic Paleustults	Fertilizer (N)	Temporary grassland	2	21.1	Noble et al 2008

Australia	-20.18	146.72	219	23.3	527	Haplustalfs	Continuous grazing	Permanent grassland/woody vegetation	4	-30.1	Northup et al 1999
Australia	-36	149	800	10	645	Granite-derived soil	Liming	Permanent grassland/sown pasture	1	-11.2	Orgill et al 2015
China	41.77	115.68	1380	1.0	360	Kasternozems	Continuous grazing	Permanent grassland	2	23.4	Pan et al 2016
China	39.13	105.58	1360	9	134	Typic Calciorthid	Grazing exclusion	Permanent grassland/shrubs	1	-13.7	Pei et al 2008
Uruguay; Argentina	-31.83 - -33.87	-55.55 - -58.28	52 – 228	16.3 – 18.9	1099 - 1406	nr	Grazing exclusion	Permanent grassland	4	-12.7	Pineiro et al 2009
Ecuador	-1.45	-78.99	4000	8.0	900	Hapludands	Continuous grazing	Permanent grassland	4	-20.2	Podwojewski et al 2002
Australia	-21.03	141.78	148	25.2	520	Vertisol	Continuous grazing	Permanent grassland	5	-5.6	Pringle et al 2014
China	43.46	116.78	1283	0.7	350	Calcic Kastanozems	Grazing exclusion	Permanent grassland	3	-16.51	Qi et al 2011
China	36.27	106.43	1974	6.9	425	Haplic Calcisols	Grazing exclusion	Permanent grassland	1	-24.5	Qiu et al 2013
Iran	31.82	50.85	2500	6.7	860	nr	Grazing exclusion	Natural grassland	1	-16.7	Raiesi and Asadi 2006
Iran	31.83	51.23	2200	10.7	255	Typic Calcixerepts	Grazing exclusion	Permanent grassland	1	-4.8	Raiesi and Riahi 2014

United Kingdom	55.5	-2.25	320	7.4	971	Brown forest soil	Liming	Semi-natural grassland	1	-3.7	Rangel-Castro et al 2004
USA	41	-104	1930	7.7	380	Aridic Argiustolls	Continuous grazing	Permanent grassland	2	48.9	Reeder and Schuman 2002
USA	40.82	-104.75	1645	8.6	325	Ustollic Haplargid	Continuous grazing	Permanent grassland	2	7.7	Reeder and Schuman 2002; Reeder et al 2004
USA	39.06 – 45.4	-93.2 - - 104.77	270 – 1650	6.3 – 12	364 - 872	Sand – Silt loam	Fertilizer (N)	Permanent grassland	5	5.3	Riggs et al 2015
USA	45.05	-110.5	1900	4.6	391	nr	Grazing exclusion	Permanent grassland	1	4.5	Risch and Frank 2006
China	46.1	87.97	723	3.5	120	Calciorthid	Grazing exclusion	Permanent grassland/shrubs	1	-34.3	Rong et al 2014
China	37.62	101.2	3200	-2	500	nr	Continuous grazing	Permanent grassland	1	-14.20	Rui et al 2011
China	43.63	116.7	1250	0.3	346	Calcic Chernozem	Grazing exclusion	Permanent grassland	4	-12.7	Sarula et al 2014
Switzerland	46.65	7.9	1925	1	1800	Acid Cambisol	Liming	Permanent grassland	1	-2.7	Scaffner et al 2012
New Zealand	-37.48	175.05	207	13	1630	Ultic Hapludand	Fertilizer (P)	Semi-natural grassland	5	2.6	Schipper et al 2011

China	43.52	116.7	1200	0.7	335	Calcic Chernozems	Continuous grazing	Permanent grassland	3	-5.3	Schonbach et al 2012
USA	41.18	-104.90	1923	7.8	384	Aridic Argiustoll	Continuous grazing	Permanent grassland	2	26.1	Schuman et al 1999
China	37.28	102.52	3600	-0.1	416	Cryrendoll	Grazing exclusion	Permanent grassland	1	35.1	Shi et al 2013
USA	42	-107.5	1591 - 2167	5.7 – 7.8	200 - 213	Ustic Haplargid	Grazing exclusion	Permanent grassland/shrubs	4	0.2	Shrestha and Stahl 2008
Germany	50.22	6.85	475	6.9	811	Pseudo gley	Liming	Permanent grassland	1	11.03	Sochorova et al 2016
France	45.77	6.58	2000	5	1600	Calcschist	Liming	Permanent grassland	1	-3.8	Spiegelberger et al 2010
China	43.63	116.7	1270	0.7	343	Calcic Chernozems	Grazing exclusion	Permanent grassland	3	-7.8	Steffens et al 2008
China	33.83	102	3500	2.2	620	Alpine meadow soil	Grazing exclusion	Permanent grassland	3	-18.4	Sun et al 2011
China	30.95	88.7	4675	0	300	Steppe soil	Grazing exclusion	Permanent grassland	1	-1.9	Sun et al 2014
South Africa	-24.75	31.37	1260	14.4	370	Aridisols	Grazing exclusion	Permanent grassland/shrubs	1	-19.34	Talore et al 2015
China	43.03	119.65	480	7.3	318	Orthi-sandic Entisols	Grazing exclusion	Permanent grassland	1	-92.4	Tang et al 2016
China	43.63	116.7	1200	1.5	350	Chestnut soil	Grazing exclusion	Permanent grassland	1	-0.7	Wang et al 2010

China	29.93	83.87	4600	-0.8	320	Cryic aridisols	Continuous grazing	Permanent grassland	3	-16.3	Wang et al 2012
China	35.95	104.15	1902	7.4	373	Sierozem	Grazing exclusion	Permanent grassland	1	-10.8	Wang et al 2014
China	42.45	116.67	1381	2.1	382	Calcic Luvisols	Fertilizer (N)	Permanent grassland	1	13.03	Wang et al 2015
China	37.93	107.0	1420	8.6	282	Aridisols	Grazing exclusion	Permanent grassland	1	-32.0	Wang et al 2016
China	30.77	90.98	4730	-0.6	290	nr	Grazing exclusion	Permanent grassland	1	18.6	Wei et al 2012
USA	41.33	-106.28	3230	5.3	1200	nr	Grazing exclusion	Permanent grassland	1	63.8	Welker et al 2004
China	38.9 – 39.08	105.6 – 105.8	1360 - 2100	4.9 – 9.2	145 - 280	Calcisols; Yermosols	Grazing exclusion	Permanent grassland	2	-6.9	Wen et al 2013
China	43.55	116.67	1200	1.1	345	Calcic Chernozems	Grazing exclusion	Permanent grassland	1	-60.0	Wen et al 2016
China	43.63	116.7	1270	0.7	350	Calcic Chernozems	Continuous grazing	Permanent grassland	2	-27.0	Wiesmeier et al 2012
China	33.75	102.07	3500	1.2	620	Alpine meadow soil	Grazing exclusion	Permanent grassland	1	-16.3	Wu et al 2010
China	31.41 – 32.3	81.82 – 92.03	4448 - 4715	-1.91 – 1.25	120 - 508	nr	Grazing exclusion	Permanent grassland	8	11.4	Wu et al 2013
China	50.21	120.75	675	-2.2	339	nr	Grazing exclusion	Permanent grassland	1	-12.35	Wu et al 2014

China	36.13	106.08	1780	6.4	448	Castanozem	Grazing exclusion	Permanent grassland	8	-41.6	Xie and Wittig 2004
China	43	116	1313	1.5	295	nr	Continuous grazing	Permanent grassland	5	-38.1	Xie and Wu 2016
China	31.60 – 31.98	91.4 – 92.4	4575 - 4680	-0.6 - -1.2	410 - 481	Cambisols	Grazing exclusion	Permanent grassland	4	-21.7	Xiong et al 2014
China	43.83	116.57	1100	-0.4	350	nr	Continuous grazing	Permanent grassland	4	-15.5	Xu et al 2007
China	41.77	115.68	1375	1.5	400	Calcic Kastanozems	Continuous grazing	Permanent grassland	3	-3.9	Xu et al 2014
China	34.9	102.1	3840	0.9	690	Alpine meadow soil	Continuous grazing	Permanent grassland	3	42.1	Yang et al 2016
China	42.97	120.72	360	6.5	366	Sand	Grazing exclusion	Permanent grassland/shrubs	1	-11.6	Yong-Zhong et al 2005
Ethiopia	4.93	38.17	1553	20	550	Cambisols	Grazing exclusion	Permanent grassland	1	-46.9	Yusuf et al 2015
China	42.03	116.27	1344	2.1	385	Calcic Luvisols	Fertilizer (N)	Permanent grassland	7	-2.2	Zhang et al 2008
China	30.05 – 30.50	91.05	4330 - 4800	1.8	479	nr	Grazing exclusion	Permanent grassland	3	-3.0	Zhao et al 2016
China	49.37	120.03	600	0	400	Calcicorthic Aridisol	Grazing exclusion	Permanent grassland	1	-14.3	Zhong et al 2014
China	41.73	116.38	1475	1.6	385	Calcic Kastanozems	Grazing exclusion	Permanent grassland	1	-36.25	Zhou et al 2007

China	36.13	106.83	1650	9.2	359	Aeolian soil	Grazing exclusion	Permanent grassland	1	-40.5	Zhu et al 2016
China	42.92	120.7	360	6.4	360	Sand	Continuous grazing	Permanent grassland/shrubs	1	-34.78	Zuo et al 2008

Nos = number of datasets, nr = not recorded, N = nitrogen, P = phosphorus, Grazing exclusion = experiments where plots excluded from grazing at the start of the experiment were used as control, Continuous grazing = experiments were previously ungrazed plots served as control plots.

Appendix 2.2: References for studies used for meta-analysis.

Table A2.2: Studies selected for meta-analysis.

Authors and year of publication	Title of article	Journal
Aldezabal et al 2015	Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland	Applied Soil Ecology 96, 251–260
Altesor et al 2006	Ecosystem changes associated with grazing in subhumid South American grasslands	Journal of Vegetation Science 17: 323-332
An and Li 2015	Effects of grazing on carbon and nitrogen in plants and soils in a semiarid desert grassland, China	J Arid Land 7(3): 341–349
Andrioli et al 2010	Influence of cattle grazing on nitrogen cycling in soils beneath Stipa tenuis, native to central Argentina	Journal of Arid Environments 74, 419–422
Aye et al 2016	The impact of long-term liming on soil organic carbon and aggregate stability in low-input acid soils	Biol Fertil Soils, 52:697–709
Bagchi and Ritchie 2010	Introduced grazers can restrict potential soil carbon sequestration through impacts on plant community composition	Ecology Letters, 13: 959–968
Bai et al 2012	Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient	Journal of Applied Ecology, 49, 1204–1215
Basher and Lynn 1996	Soil changes associated with cessation of sheep grazing in the Canterbury high country, New Zealand	New Zealand Journal of Ecology, 20(2): 179-189
Boddy et al 2007	Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils	Soil Biology & Biochemistry 39, 827–835

Cao et al 2013	Effects of grazing intensity on soil labile organic carbon fractions in a desert steppe area in Inner Mongolia	Proceedings of the International Conference on Combating Land Degradation in Agricultural Areas (ICCLD'10) Zi'An City, PR China. 11-15 October 2010. http://www.springerplus.com/content/2/S1/S1
Cardoso et al 2010	Carbon and nitrogen stocks in soil in native forests and pasture in the Pantanal biome, Brazil	Pesq. agropec. bras., Brasília, 45 (9), 1028-1035
Chen and Tang 2016	Long-term grazing exclusion effects on vegetation characteristics, soilproperties and bacterial communities in the semi-arid grasslands ofChina	Ecological Engineering 97 (2016) 170–178
Chen et al 2008	Responses of soil respiration to simulated precipitation pulses in semiarid steppe under different grazing regimes	Journal of Plant Ecology, 1, (4), 237–246
Chen et al 2012	Effects of Grazing Exclusion on Soil Properties and on Ecosystem Carbon and Nitrogen Storage in a Sandy Rangeland of Inner Mongolia, Northern China	Environmental Management, 50:622–632
Cheng et al 2016	Long-term grazing exclusion effects on vegetation characteristics, soilproperties and bacterial communities in the semi-arid grasslands ofChina	Ecological Engineering 97, 170–178
Cui et al 2005	Effect of long-term grazing on soil organic carbon content in semiarid steppes in Inner Mongolia	Ecol Res, 20: 519–527
Curtin et al 2007	Sulphur in soil and light fraction organic matter as influenced by long-term application of superphosphate	Soil Biology & Biochemistry 39, 2547–2554
da Silva et al 2014	Soil carbon indices as affected by 10 years of integrated crop–livestock production with different pasture grazing intensities in Southern Brazil	Agriculture, Ecosystems and Environment 190, 60–69

Damien et al 2015	How does soil particulate organic carbon respond to grazing intensity in permanent grasslands?	Plant Soil, 394:239–255
Deng et al 2014	Long-term fencing effects on plant diversity and soil properties in China	Soil & Tillage Research 137, 7–15
Derner et al 2006	Grazing and ecosystem carbon storage in the North American Great Plains	Plant and Soil, 280:77–90
Dong et al 2012	Response of soil properties to yak grazing intensity in a Kobresia parva-meadow on the Qinghai–Tibetan Plateau, China	Journal of Soil Science and Plant Nutrition, 2012, 12 (3) 535-546
Evans et al 2012	Long-term grazing effects on grassland soil properties in southern British Columbia	Can. J. Soil Sci. (2012) 92: 685693
Fan et al 2013	Effects of Grazing and Fencing on Carbon and Nitrogen Reserves in Plants and Soils of Alpine Meadow in the three Headwater Resource Regions	Russian Journal of Ecology, 44, 80–88.
Fang et al 2014	Nitrogen deposition impacts on the amount and stability of soil organic matter in an alpine meadow ecosystem depend on the form and rate of applied nitrogen	European Journal of Soil Science, 65, 510–519
Fornara and Tilman 2012	Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition	Ecology, 93(9), 2030–2036
Fornara et al 2013	Multi-nutrient vs. nitrogen-only effects on carbon sequestration in grassland soils	Global Change Biology, 19, 3848–3857,
Fuhlendorf et al 2002	Effects of Grazing on Restoration of Southern Mixed Prairie Soils	Restoration Ecology 10 (2), 401–407
Garcia and Nahas 2007	Biomass and microbial activity in pasture soil under different sheep grazing pressure	R. Bras. Ci. Solo, 31, 269-276

Gill 2007	Influence of 90 Years of Protection from Grazing on Plant and Soil Processes in theSubalpine of the Wasatch Plateau, USA	Rangeland Ecology & Management, 60, 88-98
Golluscio et al 2009	Sheep Grazing Decreases Organic Carbon and Nitrogen Pools in the Patagonian Steppe: Combination of Direct and Indirect Effects	Ecosystems, 12, 686–697
Gong et al 2014	Response of carbon dioxide emissions to sheep grazing and N application in an alpine grassland – Part 2: Effect of N application	Biogeosciences, 11, 1751–1757.
Gudmundsson et al 2004	Organic carbon accumulation and pH changes in an Andic Gleysol under a long-term fertilizer experiment in Iceland	Catena 56, 213–224
Guo et al 2016	Soil phosphorus fractions and arbuscular mycorrhizal fungi diversity following long-term grazing exclusion on semi-arid steppes in Inner Mongolia	Geoderma 269, 79–90
Hafner et al 2012	Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by $^{13}CO_2$ pulse labeling	Global Change Biology, 18, 528–538
Harris et al 2007	Fire and grazing in grasslands of the Argentine Caldenal: Effects on plant and soil carbon and nitrogen	Acta Oecologica, 32, 207 – 214
He et al 2008	Carbon and nitrogen store and storage potential as affected by land-use in a Leymus chinensis grassland of northern China	Soil Biology & Biochemistry 40, 2952–2959
He et al 2009	Changes in carbon and nitrogen in soil particle-size fractions along a grassland restoration chronosequence in northern China	Geoderma 150, 302–308
He et al 2011	Grazing intensity impacts soil carbon and nitrogen storage	Ecosphere 2(1):art8. doi:10.1890/ES10-00017.1

	of continental steppe	
He et al 2012	Land-use impact on soil carbon and nitrogen sequestration in typical steppe ecosystems, Inner Mongolia	J. Geogr. Sci., 22(5): 859-873
He et al 2013	Enhancement of Carbon Sequestration in Soil in the Temperature Grasslands of Northern China by Addition of Nitrogen and Phosphorus	PLoS ONE 8(10): e77241. doi:10.1371/journal.pone.0077241
Hewins et al 2015	Grazing, regional climate and soil biophysical impacts on microbial enzyme activity in grassland soil of western Canada	Pedobiologia 58, 201–209
Hofstede 1995	The effects of grazing and burning on soil and plant nutrient concentrations in Colombian p,Aramo grasslands	Plant and Soil 173: 111-132
Hopkins et al 2009	Soil organic carbon contents in long-term experimental grassland plots in the UK (Palace Leas and Park Grass) have not changed consistently in recent decades	Global Change Biology, 15, 1739–1754
Hou et al 2014	The response of grassland productivity, soil carbon content and soil respiration rates to different grazing regimes in a desert steppe in northern China	The Rangeland Journal, 36, 573–582
Jackson et al 2015	Nitrous oxide emissions from cool-season pastures under managed grazing	Nutr Cycl Agroecosyst 101, 365–376
Jones et al 2006	Carbon sequestration in a temperate grassland; management and climatic controls	Soil Use and Management, 22, 132–142
Kemmitt et al 2006	pH regulation of carbon and nitrogen dynamics in two agricultural soils	Soil Biology & Biochemistry 38, 898–911
Kotze et al 2013	Rangeland management impacts on the properties of clayey soils along grazing gradients in the semi-arid grassland biome of South Africa	Journal of Arid Environments 97, 220e229

Krzic et al 2014	Long-term grazing effects on rough fescue grassland soils in southern British Columbia	Can. J. Soil Sci. 94, 337345
Li et al 2008	Influence of historic sheep grazing on vegetation and soil properties of a Desert Steppe in Inner Mongolia	Agriculture, Ecosystems and Environment 128, 109–116
Li et al 2011	Effects of grazing and livestock exclusion on soil physical and chemical properties in desertified sandy grassland, Inner Mongolia, northern China	Environ Earth Sci, 63, 771–783
Li et al 2012a	Changes in soil C, N, and P with long-term (58 years) cattle grazing on rough fescue grassland	J. Plant Nutr. Soil Sci. 175, 339–344
Li et al 2012b	Effects of grazing exclusion on carbon sequestration and the associated vegetation and soil characteristics at a semi-arid desertified sandy site in Inner Mongolia, northern China	Can. J. Soil Sci. 92, 807-819
Li et al 2014a	Effects of Nitrogen and Phosphorus Fertilization on Soil Carbon Fractions in Alpine Meadows on the Qinghai-Tibetan Plateau	PLoS ONE, 9(7): e103266. doi:10.1371/journal.pone.0103266
Li et al 2014 b	Effects of fencing on vegetation and soil restoration in a degraded alkaline grassland in northeast China	J Arid Land, 6(4), 478–487
Liu et al 2011	Grazing intensity effects on soil nitrogen mineralization in semi-arid grassland on the Loess Plateau of northern China	Nutr Cycl Agroecosyst 91, 67–75
Liu et al 2012	Impact of Grazing on Soil Carbon and Microbial Biomass in Typical Steppe and Desert Steppe of Inner Mongolia	PLoS ONE 7(5): e36434. doi:10.1371/journal.pone.0036434
Liu et al 2016	Effects of pasture management on soil fertility and microbial communities in the semi-arid grasslands of Inner Mongolia	J Soils Sediments 16, 235–242
Ljubicic et al 2014	Plant diversity and chemical soil composition of rocky pastures in relation to the sheep grazing intensity on	Acta Bot. Croat. 73 (2), 419–435

	the northern Adriatic islands (Croatia)	
Lu et al 2015a	Carbon, nitrogen, and phosphorus storage in alpine grassland ecosystems of Tibet: effects of grazing exclusion	Ecology and Evolution 5(19), 4492–4504
Lu et al 2015b	Short-term grazing exclusion has no impact on soil properties and nutrients of degraded alpine grassland in Tibet, China	Solid Earth, 6, 1195–1205
Luan et al 2014	Different grazing removal exclosures effects on soil C stocks among alpine ecosystems in east Qinghai–Tibet Plateau	Ecological Engineering 64, 262–268
Ma et al 2016	Comparison of soil carbon and nitrogen stocks at grazing-excludedand yak grazed alpine meadow sites in Qinghai–Tibetan Plateau, China	Ecological Engineering 87, 203–211
Maillard et al 2015	Carbon accumulates in organo-mineral complexes after long-term liquid dairy manure application	Agriculture, Ecosystems and Environment 202, 108–119
Malhi et al 1997	Increasing organic C and N in soil under bromegrass with long-term N fertilization	Nutrient Cycling in Agroecosystems 49, 255–260,
Malhi et al 2003	Total and light fraction organic C in a thin Black Chernozemic grassland soil as affected by 27 annual applications of six rates of fertilizer N	Nutrient Cycling in Agroecosystems 66, 33–41
Maly et al 2009	Effects of long-term mineral fertilization on microbial biomass, microbial activity, and the presence of r- and K-strategists in soil	Biol Fertil Soils 45, 753–760
Martinsen et al 2011	Carbon storage in low-alpine grassland soils: effects of different grazing intensities of sheep	European Journal of Soil Science, 62, 822–833
McIntosh et al 1997	Effects of Exclosure and Management on Biomass and Soil Nutrient Pools in Seasonally Dry High Country, New Zealand	Journal of Environmental Management 51, 169–186

Medina-Roldan et al 2012	Grazing exclusion affects soil and plant communities, but has no impact on soil carbon storage in an upland grassland	Agriculture, Ecosystems and Environment 149, 118– 123
Mekuria et al 2011	Restoration of Ecosystem Carbon Stocks Following Exclosure Establishment in Communal Grazing Lands in Tigray, Ethiopia	Soil Sci. Soc. Am. J. 75, 246–256
Mijangos et al 2010	Effects of liming on soil properties and plant performance of temperate mountainous grasslands	Journal of Environmental Management 91, 2066-2074
Neff et al 2002	Variable effects of nitrogen additions on the stability and turnover of soil carbon	Nature, 419, 915 – 917
Ngatia et al 2015	The effects of herbivory and nutrients on plant biomass and carbon storage in Vertisols of an East African savanna	Agriculture, Ecosystems and Environment 208, 55–63
Noble et al 2008	Soil acidification and carbon storage in fertilized pastures of Northeast Thailand	Geoderma 144, 248–255
Northup et al 1999	Grazing impacts on the spatial distribution of soil microbial biomass around tussock grasses in a tropical grassland	Applied Soil Ecology 13, 259 – 270
Orgill et al 2015	Soil carbon under perennial pastures; benchmarking the influence of pasture age and management	IOP Conf. Series: Earth and Environmental Science 25 (2015) 012018 doi:10.1088/1755-1315/25/1/012018
Pan et al 2016	Non-growing season soil CO2 efflux patterns in five land-use types in northern China	Atmospheric Environment 144, 160 -167
Pei et al 2008	Changes in soil properties and vegetation following exclosure and grazing in degraded Alxa desert steppe of Inner Mongolia, China	Agriculture, Ecosystems and Environment 124, 33–39
Pineiro et al 2009	Grazing effects on belowground C and N stocks along a network of cattle exclosures in temperate and subtropical grasslands of South America	Global Biogeochemical Cycles, 23, GB2003, doi:10.1029/2007GB003168

Podwojewski et al 2002	Overgrazing effects on vegetation cover and properties of volcanic ash soil in the paÂramo of Llangahua and La Esperanza (Tungurahua, Ecuador)	Soil Use and Management 18, 45-55
Pringle et al 2014	The effect of pasture utilization rate on stocks of soil organic carbon and total nitrogen in a semi-arid tropical grassland	Agriculture, Ecosystems and Environment 195, 83–90
Qi et al 2011	Effects of livestock grazing intensity on soil biota in a semiarid steppe of Inner Mongolia	Plant Soil 340,117–126
Qiu et al 2013	Ecosystem Carbon and Nitrogen Accumulation after Grazing Exclusion in Semiarid Grassland	PLoS ONE 8(1): e55433. doi:10.1371/journal.pone.0055433
Raiesi and Asadi 2006	Soil microbial activity and litter turnover in native grazed and ungrazed rangelands in a semiarid ecosystem	Biol Fertil Soils 43, 76–82
Raiesi and Riahi 2014	The influence grazing exclosure on soil C and dynamics, and ecological indicators in upland arid and semi-arid rangelands	Ecological Indicators 41, 145–154
Rangel-Castro et al 2004	Carbon flow in an upland grassland: effect of liming on the flux of recently photosynthesized carbon to rhizosphere soil	Global Change Biology 10, 2100–2108
Reeder and Schuman 2002	Influence of livestock grazing on C sequestration in semi-arid mixed grass and short-grass rangelands	Environmental Pollution 116, 457–463
Reeder et al 2004	Response of Organic and Inorganic Carbon and Nitrogen to Long-Term Grazing of the Shortgrass Steppe	Environmental Management 33, 485–495
Riggs et al 2015	Nitrogen addition changes grassland soil organic matter decomposition	Biogeochemistry 125, 203–219
Risch and Frank 2006	Carbon dioxide fluxes in a spatially and temporally heterogeneous temperate grassland	Oecologia 147, 291–302

Rong et al 2014	Effectiveness of exclosures for restoring soils and vegetation degraded by overgrazing in the Junggar Basin, China	Grassland Science, 60, 118–124
Rui et al 2011	Warming and grazing affect soil labile carbon and nitrogen pools differently in an alpine meadow of the Qinghai–Tibet Plateau in China	J Soils Sediments 11, 903–914
Sarula et al 2014	Carbon Storage under Different Grazing Management in the Typical Steppe	Eurasian Soil Science 47 (11), 1152–1160
Scaffner et al 2012	Calcium Induces Long-Term Legacy Effects in a Subalpine Ecosystem	PLoS ONE 7(12): e51818. doi:10.1371/journal.pone.0051818
Schipper et al 2011	Trends in soil carbon and nutrients of hill-country pastures receiving different phosphorus fertilizer loadings for 20 years	Biogeochemistry 104, 35–48
Schonbach et al 2012	Grazing effects on the greenhouse gas balance of a temperate steppe ecosystem	Nutr Cycl Agroecosyst 93, 357–371
Schuman et al 1999	Impact of Grazing Management on the Carbon and Nitrogen Balance of a Mixed-GrassRangeland	Ecological Applications, 9, 65-71
Shi et al 2013	Grazing exclusion decreases soil organic C storage at an alpine grassland of the Qinghai–Tibetan Plateau	Ecological Engineering 57, 183– 187
Shrestha and Stahl 2008	Carbon accumulation and storage in semi-arid sagebrush steppe: Effects of long-term grazing exclusion	Agriculture, Ecosystems and Environment 125, 173–181
Sochorova et al 2016	Long-term agricultural management maximizing hay production can significantly reduce belowground C storage	Agriculture, Ecosystems and Environment 220, 104–114
Spiegelberger et al 2010	Resilience of acid subalpine grassland to short-term liming and fertilisation	Agriculture, Ecosystems and Environment, 137 (1-2), 162.
Steffens et al 2008	Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China)	Geoderma 143, 63–72

Sun et al 2011	Grazing depresses soil carbon storage through changing plant biomass and composition in a Tibetan alpine meadow	Plant Soil Environ., 57, (6), 271–278
Sun et al 2014	Effects of Grazing Regimes on Plant Traits and Soil Nutrients in an Alpine Steppe, Northern Tibetan Plateau	PLoS ONE 9(9): e108821. doi:10.1371/journal.pone.0108821
Talore et al 2015	Long-term impacts of season of grazing on soil carbon sequestration and selected soil properties in the arid Eastern Cape, South Africa	Plant Soil 397, 317–329
Tang et al 2016	Effects of excluding grazing on the vegetation and soils of degraded sparse-elm grassland in the Horqin Sandy Land, China	Agriculture, Ecosystems and Environment 235, 340–348
Wang et al 2010	Effects of grazing exclusion on soil net nitrogen mineralization and nitrogen availability in a temperate steppe in northern China	Journal of Arid Environments 74, 1287e1293
Wang et al 2012	Impact of historic grazing on steppe soils on the northern Tibetan Plateau	Plant Soil 354, 173–183
Wang et al 2014	Grazing exclusion effects on above- and below-ground C and N pools of typical grassland on the Loess Plateau (China)	Catena 123, 113–120
Wang et al 2015	Nitrogen addition and mowing affect microbial nitrogen transformations in a C4 grassland in northern China	European Journal of Soil Science, 66, 485–495
Wang et al 2016	Grazing exclusion significantly improves grassland ecosystem C and N pools in a desert steppe of Northwest China	Catena 137, 441–448
Wei et al 2012	Responses of CO2, CH4 and N2O fluxes to livestock exclosure in an alpine steppe on the Tibetan Plateau, China	Plant Soil 359, 45–55

Welker et al 2004	Alpine Grassland CO2 Exchange and Nitrogen Cycling: Grazing History Effects, Medicine Bow Range, Wyoming, U.S.A.	Arctic, Antarctic, and Alpine Research, 36, 11–20
Wen et al 2013	Experimental investigation on soil carbon, nitrogen, and their components under grazing and livestock exclusion in steppe and desert steppe grasslands, Northwestern China	Environ Earth Sci 70, 3131–3141
Wen et al 2016	Dynamics of Soil Organic Carbon and Aggregate Stability with Grazing Exclusion in the Inner Mongolian Grasslands	PLoS ONE 11(1), e0146757. doi:10.1371/journal.pone.0146757
Wiesmeier et al 2012	Short-term degradation of semiarid grasslands—results from a controlled grazing experiment in Northern China	J. Plant Nutr. Soil Sci. 175, 434–442
Wu et al 2008	Storage and Dynamics of Carbon and Nitrogen in Soil after Grazing Exclusion in Leymus chinensis Grasslands of Northern China	J. Environ. Qual. 37:663–668
Wu et al 2010	Long-term fencing improved soil properties and soil organic carbon storage in an alpine swamp meadow of western China	Plant Soil 332, 331–337
Wu et al 2013	Grazing-Exclusion Effects on Aboveground Biomass and Water-Use Efficiency of Alpine Grasslands on the Northern Tibetan Plateau	Rangeland Ecol Manage 66, 454–461
Wu et al 2014	Restoration of ecosystem carbon and nitrogen storage and microbial biomass after grazing exclusion in semi-arid grasslands of Inner Mongolia	Ecological Engineering 73, 395–403
Xie and Wittig 2004	The impact of grazing intensity on soil characteristics of Stipa grandis and Stipa bungeana steppe in northern China (autonomous region of Ningxia)	Acta Oecologica 25, 197–204

Xie and Wu 2016	Effects of grazing intensity on soil organic carbon of rangelands in Xilin Gol League, Inner Mongolia, China	J. Geogr. Sci., 26(11), 1550-1560
Xiong et al 2014	Effects of Grazing Exclusion on Plant Productivity and Soil Carbon, Nitrogen Storage in Alpine Meadows in Northern Tibet, China	Chin. Geogra. Sci. 24 (4) 488–498
Xu et al 2007	The pattern between nitrogen mineralization and grazing intensities in an Inner Mongolian typical steppe	Plant Soil 300, 289–300
Xu et al 2014	Response of Vegetation and Soil Carbon and Nitrogen Storage to Grazing Intensity in Semi-Arid Grasslands in the Agro-Pastoral Zone of Northern China	PLoS ONE 9(5): e96604. doi:10.1371/journal.pone.0096604
Yang et al 2016	Soil properties and species composition under different grazing intensity in an alpine meadow on the eastern Tibetan Plateau, China	Environ Monit Assess 188, 678
Yong-Zhong et al 2005	Influences of continuous grazing and livestock exclusion on soil properties in a degraded sandy grassland, Inner Mongolia, northern China	Catena 59, 267–278
Yusuf et al 2015	Managing Semi-Arid Rangelands for Carbon Storage: Grazing and Woody Encroachment Effects on Soil Carbon and Nitrogen	PLoS ONE 10(10): e0109063. doi:10.1371/journal.pone.0109063
Zhang et al 2008	Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China	Plant Soil 311, 19–28
Zhao et al 2016	Effect of grazing exclusion on ecosystem respiration among three different alpine grasslands on the central Tibetan Plateau	Ecological Engineering 94, 599–607
Zhong et al 2014	Effects of grazing on N2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China	Soil Biology & Biochemistry 69, 1-10

Zhou et al 2007	Soil carbon and nitrogen stores and storage potential as affected by land-use in an agro-pastoral ecotone of northern China	Biogeochemistry 82, 127–138
Zhu et al 2016	Effects of grazing exclusion on plant community and soil physicochemical properties in a desert steppe on the Loess Plateau, China	Ecological Engineering 90, 372–381
Zuo et al 2008	Spatial pattern and heterogeneity of soil properties in sand dunes under grazing and restoration in Horqin Sandy Land, Northern China	Soil & Tillage Research 99, 202–212

Appendix 2.3: Regression model used to estimate missing bulk density values in Chapter 2.

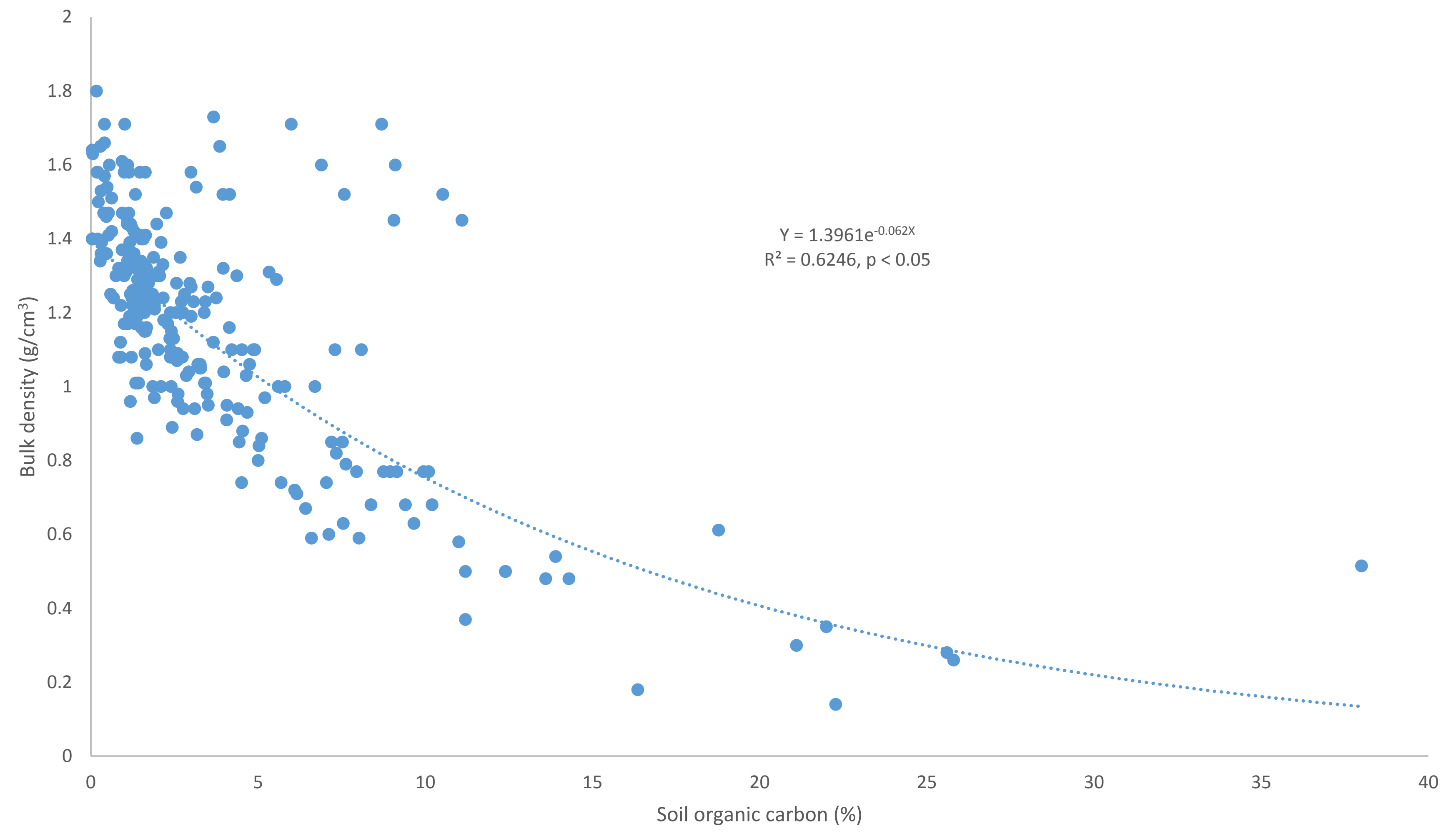

Figure A2.1: The relationship between SOC (%) and bulk density (g/cm^{-3})

Appendix 4.1: Carbon flux results from different European grasslands

Table A4.1: Reported CO_2 fluxes (g CO_2 m^{-2} $year^{-1}$; Gross primary productivity – GPP, Ecosystem respiration – ER, Net ecosystem exchange – NEE) in some European grasslands and their site and management characteristics.

Country	Elevation (m)	MAT (°C)	MAP (mm)	Soil type	Grassland management activities	FM duration (years)	GPP	ER	NEE	Author
The Netherlands	7	9.5	760	Alluvial clay	Cut (litter not removed)	1	5915	3521	-2394	Gilmanov *et al.*, 2007
Italy	1699	5.5	1816	Sandy loam	Grazed; 45 kg N/ha	1	3972	2346	-1626	Gilmanov *et al.*, 2007
UK	190	9.0	870	Sandy-clay loam	Cut and grazed;147 kg N/ha	1	6793	5451	-1324	Gilmanov *et al.*, 2007
Ireland	50	10.1	974	Luvisol	Cut and grazed; 200 kg N/ha	1	6807	5622	-1185	Gilmanov *et al.*, 2007
Denmark	15	8.5	1119	Sandy loam	Cut; 176 kg N/ha	1	6873	5730	-1143	Gilmanov *et al.*, 2007
Italy	900	9.5	1243	Haplic phaeozem	Cut and grazed	1	4778	3994	-784	Gilmanov *et al.*, 2007
Switzerland	450	9.2	1109	Stagnic cambisol	Cut	2	5123	4426	-697	Gilmanov *et al.*, 2007

Germany	385	7.2	853	Pseudo-gley	Cut	1	4742	4206	-536	Gilmanov *et al.*, 2007
The Netherlands	0	10	780	Clay polder	Cut and grazed; 237 kg N/ha	1	3974	3448	-526	Gilmanov *et al.*, 2007
France	1040	8.62	1013	Andosol	Grazed; 170 kg N/ha	2	4649	4160	-509	Gilmanov *et al.*, 2007
Ireland	195	9.4	1207	Gleysol	Cut; 175 – 350 kg N/ha	6	1738	1442	-277	Peichl *et al.*, 2011
Italy	1550	5.5	1189	Humic umbrisol	Cut	1	4527	4253	-274	Gilmanov *et al.*, 2007
France	1040	8.62	1064	Andosol	Grazed	2	4418	4193	-225	Gilmanov *et al.*, 2007
UK	190	9.0	947	Eutri cambisol	Cut and grazed; 168 – 224 kg N/ha	2	1756	1538	-218	Jones *et al.*, 2017
The Netherlands	7	9.2	740	Eutric gleyic fluvisol	Cut	4	2011	1803	-208	Jacobs *et al.*, 2007
Hungary	248	8.9	750	Alfisol	Cut	2	5867	5668	-199	Gilmanov *et al.*, 2007
Spain	1770	6.1	1064	Lithic cryrendoll	Grazed	2	2221	2049	-172	Gilmanov *et al.*, 2007

UK	350-400	5.9-7.7	1002-1131	Podzol	Grazed	1	745	584	-161	Quin *et al.*, 2015
Austria	970	6.5	852	Fluvisol	Cut	1	5748	5726	-22	Gilmanov *et al.*, 2007
Germany	62	9.0	560	Haplic albeluvisol	Cut and fertilized (fertilizer rates not given)	1	1779 - 2153	2052 - 2204	-101 to +425	Huth *et al.*, 2017
Austria	970	6.5	852	Fluvisol	Cut	7	1568	1586	+18	Wohlfahrt *et al.*, 2008
Hungary	111	9.8	450	Sandy chernozem	Grazed	1	1713	1809	+96	Gilmanov *et al.*, 2007
Finland	104	3.9	581	Terric histosol	Cut	1	2486	2642	+156	Gilmanov *et al.*, 2007
Portugal	190	14.6	387	Luvisol	Grazed	2	1936	2200	+264	Gilmanov *et al.*, 2007
Switzerland	1025	7.3	1327	Stagnic cambisol	Cut and grazed	1	5320	5927	+627	Gilmanov *et al.*, 2007

Grazed = grassland is grazed by livestock, Cut = grassland vegetation is cut and removed, FM = flux measurement.

Appendix 4.2: Modelled relationships between measured carbon flux results and environmental variables

Table A4.2: A multiple linear regression result of measured CH_4 flux as a function of soil moisture and temperature for one collar in Nidderdale (n = 12).

	Coefficients	Standard Error	F Stat	t Stat	P-value
Regression model			0.06		0.946
Adjusted R square	-0.207				
Intercept	-4.117	11.00		-0.37	0.717
Soil moisture	0.005	0.16		0.03	0.977
Soil temperature	0.098	0.32		0.31	0.766

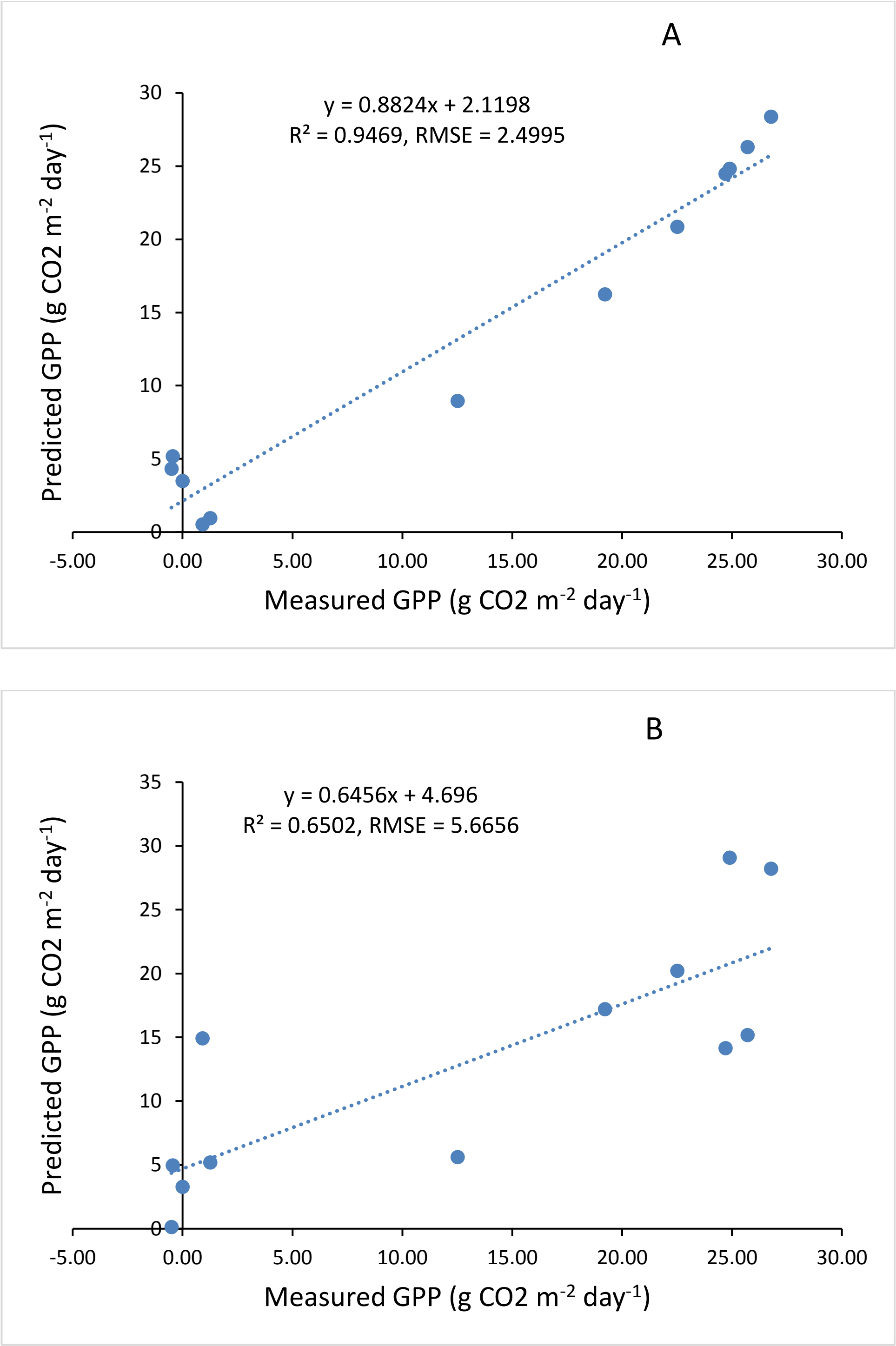

Figure A4.1: An example of linear relationships between measured GPP and the GPP predicted with Equation 4 (A) and Equation 5 (B) using data from one Nidderdale collar.

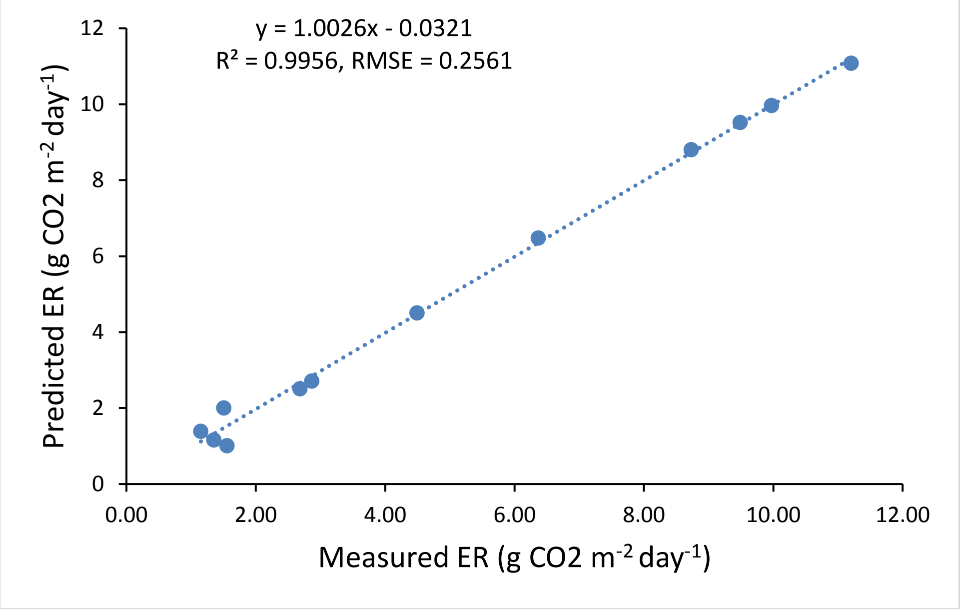

Figure A4.2: An example of linear relationships between measured ER and the predicted values (using data from one Nidderdale collar).

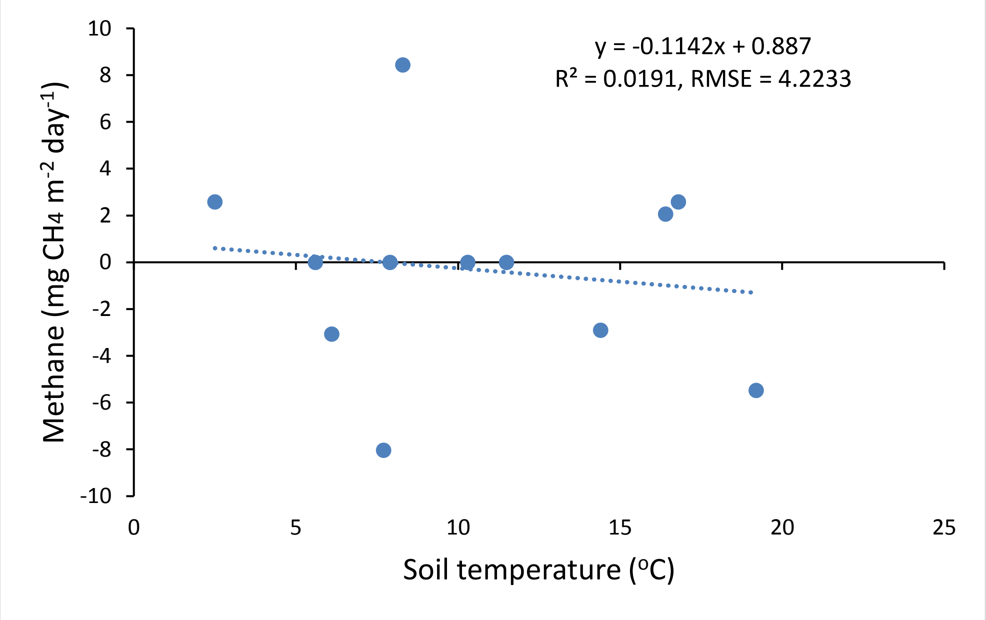

Figure 4.3: An example plot of measured methane flux as a function of soil temperature for one Nidderdale collar.

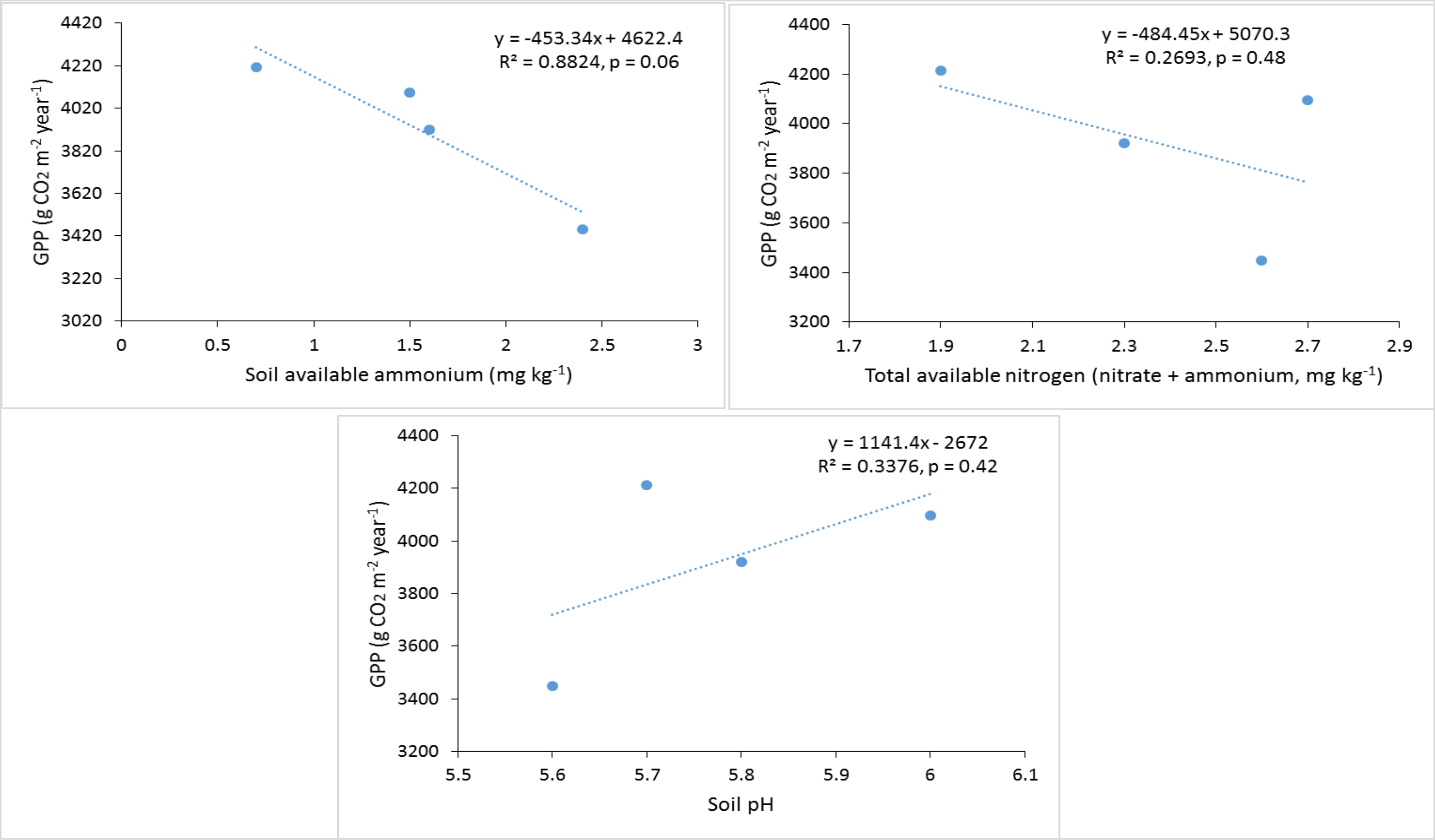

Figure A4.4: Relationships between annual gross primary productivity (GPP) and soil available ammonium, total available nitrogen and pH.

Appendix 5.1: Results of experimental climate change studies in different European grasslands

Table A5.1: Reported responses of plant biomass and C fluxes to experimental warming and altered precipitation in some European grasslands and their site characteristics.

Location	Altitude (m)	MAT (°C)	MAP (mm)	Soil	Exp. set-up	Trt type	Trt	Duration (years)	Effect on GPP	Effect on ER	Effect on AGB	Effect on BGB	Effect on DOC	Authors
Belgium		9.6	776	Silt loam	Soil cores in sun-lit chambers	W	+3.0 °C	2	-12%	-7% AGR	-30%			De Boeck *et al.*, 2007
Denmark		8.0	600	Sandy loam	Field plots	W	+1.0 °C	1			+10%			Andresen *et al.*, 2009
Denmark		8.0	600	Sandy loam	Field plots	W	+1.0 °C	3			+5%			Kongstad *et al.*, 2012
France	850	8.7	780	Cambisol	Field monoliths	W	+3.5 °C	3			+7%			Bloor *et al.*, 2010
UK (England)	370	8.0	1300	Limestone derived	Field plots	W	+3.0 °C	13			-9%			Grime *et al.*, 2008
UK (England)	150	10.0	680	Limestone derived	Field plots	W	+3.0 °C	5			+2%			Grime *et al.*, 2000
UK (Scotland)	309	8.0	900	Brown earth	Soil cores in exp. garden	W	+3.5 °C	2		+49%	-15%	+37%		Briones *et al.*, 2009
UK (England)	150	10.0	680	Limestone derived	Field plots	P	+20% of summer ppt	5			+33%			Grime *et al.*, 2000
UK (England)	370	8.0	1300	Limestone derived	Field plots	P	+26% of summer ppt	13			-3%			Grime *et al.*, 2008

Denmark		10.0	707	Loamy sand	Field plots	D	-7% of MAP	3	-11% SR			Selsted *et al.*, 2012
Denmark		8.0	600	Sandy loam	Field plots	D	-8% of MAP	3		-5%		Kongstad *et al.*, 2012
UK (England)	150	10.0	680	Limestone derived	Field plots	D	-100% of July – August ppt	5		-6%		Grime *et al.*, 2000
UK (England)	370	8.0	1300	Limestone derived	Field plots	D	-77% of summer ppt	13		-34%		Grime *et al.*, 2008
Switzerland	393	9.8	1232	Cambisol (loamy clay)	Field plots	D	-33% of MAP	1	-17% SR		-58%	Hagedorn and Joos, 2014
Switzerland	982	7.7	1765	Cambisol (loamy clay)	Field plots	D	-33% of MAP	1	-24% SR		-42%	Hagedorn and Joos, 2014
Switzerland	1978	2.3	969	Leptosol (sandy loam)	Field plots	D	-26% of MAP	1	-38% SR		-81%	Hagedorn and Joos, 2014
Switzerland	393	9.6	1103	Cambisol	Field plots	D	-31% of MAP	3		-22%		Prechsl *et al.*, 2015
Switzerland	1978	1.7	948	Cambisol	Field plots	D	-26% of MAP	2		-42%		Prechsl *et al.*, 2015
UK (England)	150	10.0	680	Limestone derived	Field plots	WP	+3.0 °C; +20% of summer ppt	5		+6%		Grime *et al.*, 2000

UK (England)	370	8.0	1300	Limestone derived	Field plots	WP	+3.0 °C; +26% of summer ppt	13		-4%	Grime *et al.*, 2008
Denmark		8.0	600	Sandy loam	Field plots	WD	+1.0 °C; -8% of MAP	3		+4%	Kongstad *et al.*, 2012
Denmark		10.0	707	Loamy sand	Field plots	WD	+0.4 °C; -7% of MAP	3	-20% SR		Selsted *et al.*, 2012
UK (England)	370	8.0	1300	Limestone derived	Field plots	WD	+3.0 °C; -77% of summer ppt	13		-25%	Grime *et al.*, 2008
UK (England)	150	10.0	680	Limestone derived	Field plots	WD	+3.0 °C; -100% of July – August ppt	5		-32%	Grime *et al.*, 2000
France	850	8.7	780	Cambisol	Field monoliths	WD	+3.5 °C; -20% of summer ppt	3		-4%	Bloor *et al.*, 2010

MAP= mean annual precipitation, MAT = mean annual temperature, Exp. = experimental, AGR = aboveground respiration, SR = soil respiration, AGB = aboveground biomass, BGB = belowground biomass, Trt = treatment, ppt = precipitation, W = warming, P= increased precipitation, D = drought, WP = warming and increased precipitation, WD = warming and drought.

Appendix 5.2: References for experimental climate change studies in different European grasslands

Table A5.2: References for the studies in Table A5.1.

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Appendix 5.3: Data used for preparing artificial rain, and modelled relationships between measured carbon flux results and variables measured under controlled environmental conditions

Table A5.3: Rainfall chemistry for Moor House – Upper Teesdale, UK (54° 41'N, 2° 23' W) from 4th June 2003 to 29th August 2012 (Rennie *et al.*, 2015). In the absence of rainfall chemistry data for our site, the artificial rain used for our experiment was prepared in the laboratory using this published rainfall chemistry data from a long-term monitoring site about 60 km away.

Ion	10-year mean (mg/L)	10-year mean (meq/L)
Ca^{2+}	0.2890	0.0144
Mg^{2+}	0.0766	0.0063
Na^{+}	0.6222	0.0271
K^{+}	0.1378	0.0035
Cl^{-}	0.9722	0.02739
SO_4^{2-}	0.9946	0.0207
NH_4^{+}	0.4885	0.0271
NO_3^{-}	0.2860	0.0046
pH 5.36		

Rennie, S., Adamson, J., Anderson, R., Andrews, C., Bater, J., Bayfield, N., Beaton, K., Beaumont, D., Benham, S., Bowmaker, V., Britt, C., Brooker, R., Brooks, D., Brunt, J., Common, G., Cooper, R., Corbett, S., Critchley, N., Dennis, P., Dick, J., Dodd, B., Dodd, N., Donovan, N., Easter, J., Eaton, E., Flexen, M., Gardiner, A., Hamilton, D., Hargreaves, P., Hatton-Ellis, M., Howe, M., Kahl, J., Lane, M., Langan, S., Lloyd, D., McElarney, Y., McKenna, C., McMillan, S., Milne, F., Milne, L., Morecroft, M., Murphy, M., Nelson, A., Nicholson, H., Pallett, D., Parry, D., Pearce, I., Pozsgai, G., Rose, R., Schafer, S., Scott, T., Sherrin, L., Shortall, C., Smith, R., Smith, P., Tait, R., Taylor, C., Taylor, M., Thurlow, M., Turner, A., Tyson, K., Watson, H., Whittaker, M., 2015. UK Environmental Change Network (ECN) precipitation chemistry data: 1992-2012. NERC Environmental Information Data Centre. https://doi.org/10.5285/0cd4abd2-1bc9-48bc-b5c2-cebdeaa23ceb.

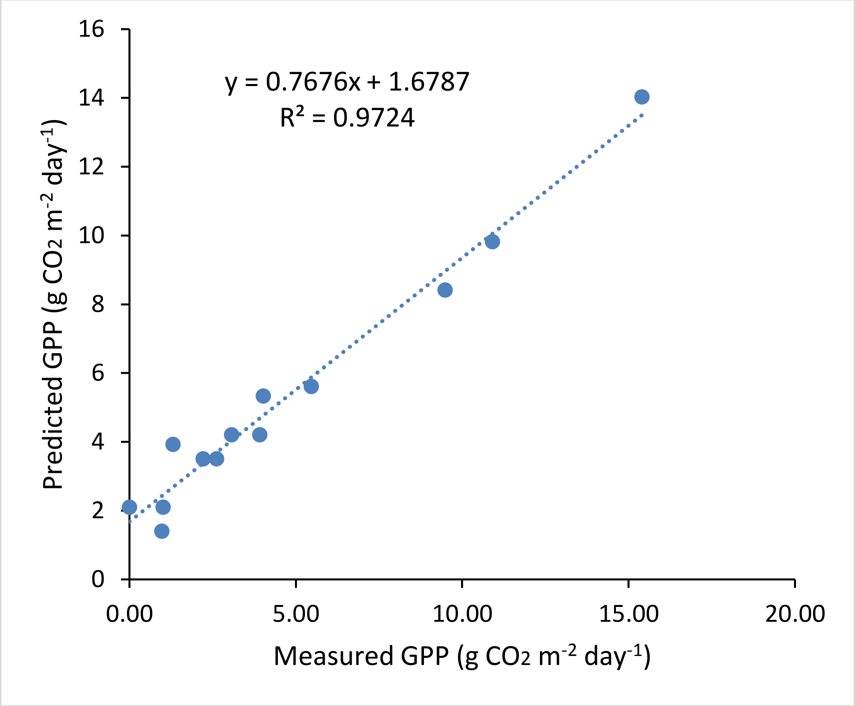

Figure A5.1: An example of linear relationships between measured and predicted GPP (using data from one soil core).

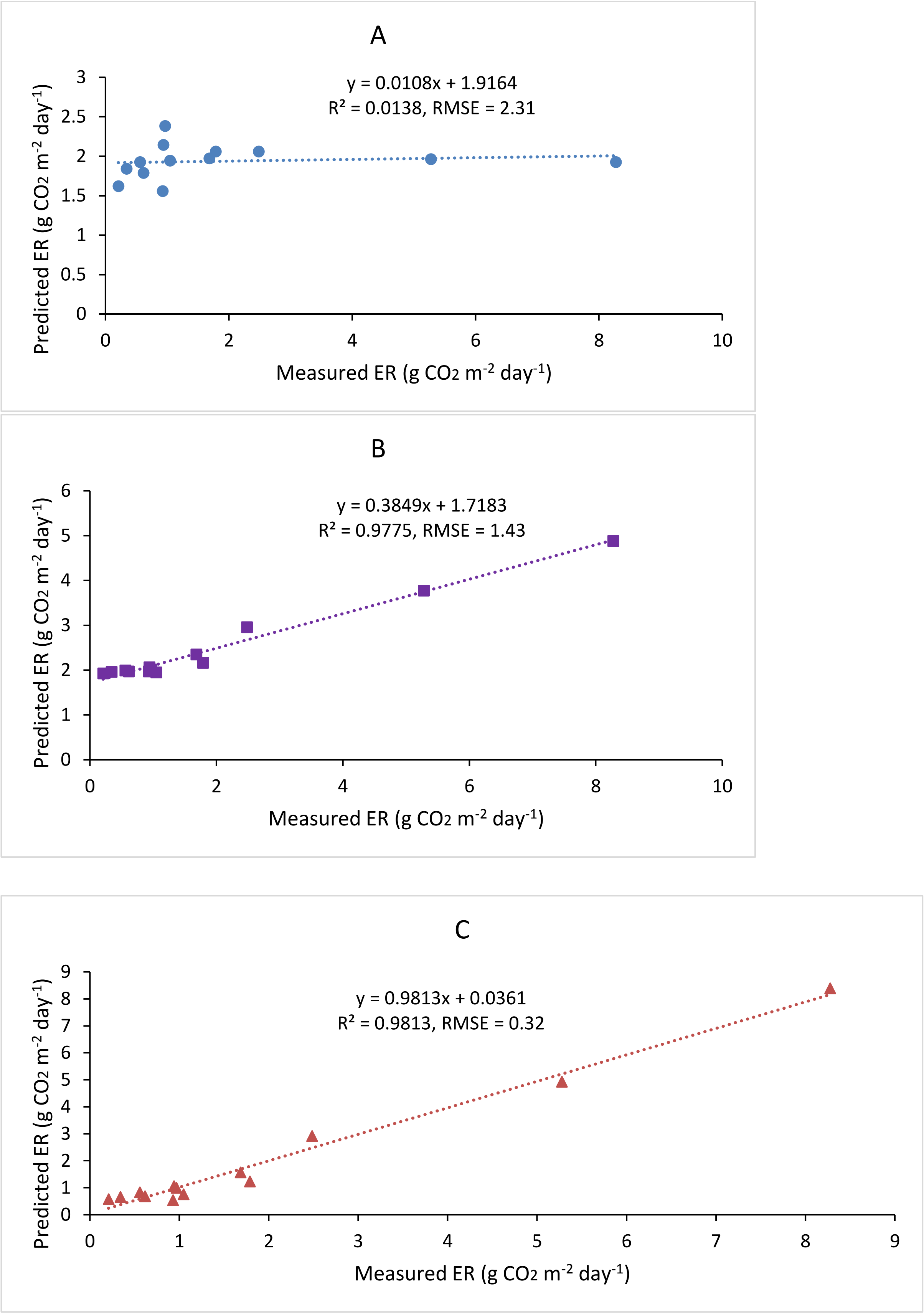

Figure A5.2: Examples of relationships between measured ER and the ER predicted using Equations 4 (A), 5 (B) and 6 (C).

Appendix 5.4: Effects of experimental climate change on carbon dioxide fluxes

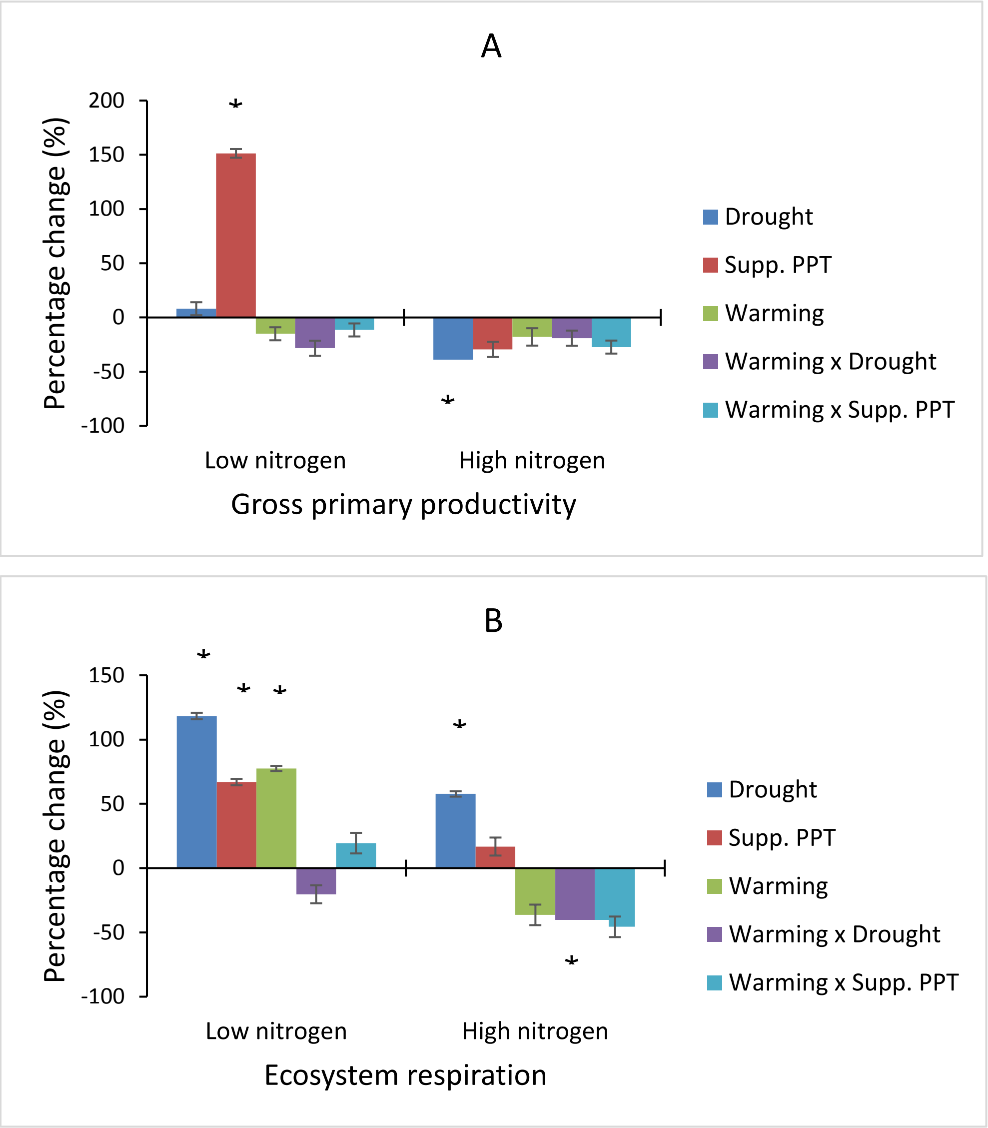

Figure A5.3: The effects of warming, drought, supplemental precipitation (Supp. PPT) and their interactions on the gross primary productivity (A) and ecosystem respiration (B) in the low nitrogen and high nitrogen cores (n = 4). Bars with asterisks represent significant change at 5% probability level in GPP or ER of soil cores subjected to altered climate treatment compared to cores with ambient climate treatment. Error bars represent standard error (n = 4).