Interactive effects of climate change and management on grassland greenhouse gas emissions

Arlete Simões Barneze

Agronomic Engineering, MSc in Science

Lancaster Environment Centre

Lancaster University

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(Agronomic Engineering, MSc in Science)

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Abstract

Climate warming has the potential to alter carbon (C) and nitrogen (N) cycling affecting greenhouse gas (GHG) emissions and a range of other ecosystem functioning in grasslands. This will be particularly important for the sustainability of agricultural ecosystems due to its role in global food security and soil C sequestration. The interaction between climate warming and grassland management is highly important and needs to be addressed as it may change the direction and strength of the effects on GHG emissions by changing plant productivity (either above and/or below-ground) and plant-soil properties. Plant species composition also plays a key role affecting the nutrient cycling thus GHG emissions in grasslands. The aim of this thesis is to understand how grassland management will influence C and N cycling under future climate change. The interactive effect of climate warming and grassland management is investigated in a field experiment over two growing seasons with varied microclimate effects, and the effect of plant composition manipulation in a controlled temperature mesocosm experiment. Overall, interactions between warming and management significantly affected GHG fluxes and plant-soil properties with important single treatment effects. The role that below-ground components plays on GHG emissions was less evident, becoming unclear the mechanisms related to gas releases to the atmosphere. Increases in legume proportions in grass-legume mixtures reduced ecosystem respiration in fertilised soils, with no effects in unfertilised soils. N cycling was not affected by increases in legume proportions. Plant productivity including above- and below-ground biomass had a non-linear relationship with relative legume proportion. Either grassland management or different plant species compositions approach may improve C sequestration and reduce GHG emissions.

Author's Declaration

I declare that this thesis has been composed by myself. It has not been accepted in any previous application for degree, the work of which has been done by myself and sources of information specifically acknowledged.

Signed:  Date: 19h December 2017

Arlete Simões Barneze

Lancaster University, December 2017

Statement of authorship

This thesis has been prepared in the alternative thesis format, as a set of four papers intended for submission to peer-reviewed journals. The papers are presented as intended for submission, except with a consolidated bibliography at the end of the thesis. All four papers have multiple authors and their contributions are detailed and certified by my supervisors below.

Chapter 2 is intended for submission as Barneze, A.S., Heath, J., Whitaker, J., McNamara, N and Ostle, N. (2018). Interactive effects of climate warming and management on temperate grassland productivity and greenhouse gas emissions. In preparation to submit to *Global Change Biology*.

A S Barneze planned the experiments with advice from N Ostle, N McNamara and J Whitaker. A S Barneze set up the field experiment with help from J. Heath. A S Barneze carried out data collection and led the data analysis and the writing of the paper, with contributions from the co-authors.

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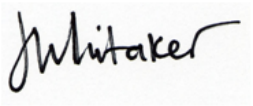
I hereby agree with the above statements:

Nicholas J Ostle Date: 19th December 2017

Niall P. McNamara Date: 19h December 2017



Jeanette Whitaker Date: 19th December 2017

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Abbreviations

AGB REMOVAL	above-ground biomass removal
AGB	above-ground biomass
AIC	akaike information criterion
AMF	arbuscular mycorrhiza
C	carbon
CH_4	methane
CO_2	carbon dioxide
d.f.	degrees of freedom
DNDC	DeNitrification DeComposition
ECD	electron capture detector
EDTA	ethylenediaminetetraacetic acid
FID	flame ionisation detector
GC	gas chromatograph
GHG	greenhouse gas
GLS	generalised least squares
GWP	global warming potential
IG	in-growth core
ITEX	International Tundra Experiment design
LCC	leaf carbon content
LDMC	leaf dry matter content
LEG	legume proportion
LEG_BIOM	legume biomass
LME	linear mixed effects
LNC	leaf nitrogen content
LRT	likelihood ratio test

N	nitrogen
N_2O	nitrous oxide
NADD	nitrogen addition
NH_3	ammonia
NH_4^+	ammonium
NH_4NO_3	ammonium nitrate
NO WARM	no warming
NO	nitric oxide
NO_2^-	nitrite
NO_3^-	nitrate
NoNADD	non-nitrogen addition
NSRI	National Soil Resources Institute
O_2	oxygen
OTC	open-top chambers
PBG	partitioned below-ground
RCC	root carbon content
RD	relative deviation
RDMC	root dry matter content
RMSE	root mean square error
RNC	root nitrogen content
SE	standard error
SLA	specific leaf area
SOC	soil organic carbon
SRL	specific root length
VIF	variance inflation factors
WARM	warming
WFPS	water-filled pore space

1 General Introduction

Grasslands are an important land-use globally, particularly due to their potential to hold very large carbon (C) stores and as a notable source of greenhouse gas (GHG) emissions. Global warming is expected to increase surface temperatures but the consequences for plant productivity (Wu et al., 2012), microclimate conditions (soil temperature and moisture) (Brzostek et al., 2012), length of growing seasons (Post et al., 2009) and biodiversity (O'Neill et al., 2017) in grassland ecosystems are still unclear. Climate change is also likely to affect C and nitrogen (N) cycling, with the magnitude of impacts and feedbacks altered under different grassland management strategies. It is therefore crucial to understand these consequences of climate change for grasslands in order to maintain or increase productivity whilst mitigating GHG emissions from soil. This thesis investigates how climate change and grassland management strategies alters nutrient cycling in soil and the release of GHGs to the atmosphere. Specifically, it considers how the interactive effect of climate warming and grassland management changes C and N cycling and CO_2, N_2O and CH_4 emissions from soils.

1.1 A changing world and grasslands

Global climate is changing, with increasing atmospheric carbon dioxide (CO_2), and an estimated increase in the average surface temperature of 1.5 to 2 °C by the end of the century (IPCC, 2014). A 1 °C increase in the average surface temperature has already been detected since the industrial revolution (WMO, 2016). Projections also point to changes in the rainfall pattern where drought and flooding could be more frequent and extreme. Increases in GHG emissions, CO_2, N_2O and CH_4 concentration, are driving these global climate changes (Lal, 2004). CO_2 is the most important anthropogenic GHG in the atmosphere, and its concentration increased 44% of the pre-industrial level (before 1750), an increase of 2.08 ppm y^{-1} (WMO, 2016). The main contributor is the combustion of fossil fuels and deforestation associated with other land-use change (Lal, 2004). Although, N_2O and CH_4 have lower concentrations in the atmosphere compared to CO_2, their global warming potential (GWP) is 298 and 34 times higher than CO_2 over a 100-year time horizon (IPCC, 2013). The concentration of CH_4 in the atmosphere has increased by 6 ppb y^{-1} and reached 156% of the pre-industrial level (WMO, 2016).

Anthropogenic sources account for 60% of emissions, including enteric fermentation in ruminants, agriculture, fossil fuel exploitation, while natural sources, such as decomposition of organic matter in wetlands, account for the remaining 40% of emissions. N_2O is the third most important anthropogenic gas and has become the prime emission breaking down and depleting stratospheric ozone (Duxbury et al., 1993). Atmospheric N_2O concentrations have also been steadily rising and increased by 21% since pre-industrial era with an average of 0.89 ppb y^{-1} (WMO, 2016). Increases in N_2O concentrations are strongly linked to anthropogenic emissions over the decades, and agriculture accounts for 56-70% of the total N_2O emissions produced by the terrestrial ecosystem (Syakila and Kroeze, 2011). Agriculture accounted for an estimated emission of 49 Mt CO_2 equivalent in 2015 in the UK, 33% of N_2O emissions, and 56% of CH_4 emissions (BEIS, 2017).

Grasslands are estimated to occupy between 20-40% of the Earth land cover (FAO, 2015), and 36% of UK land cover area (Carey et al., 2008). Grasslands, as one of the main global ecosystems, are under pressure by these global changes and anthropogenic activities (Gibson, 2008). The pressures include increases in food production to feed the unprecedented population growth (Lutz and KC, 2010), and to deliver concomitant ecosystem services via sustainable management intensification (Garnett et al., 2013).

Among the ecosystem services provided by grasslands, soil C storage is particularly important as, for example, temperate grasslands store approximately 304 Pg C, corresponding to 12% of global C (Read et al., 2001). However, grasslands can be a source of GHG emissions, especially N_2O, which is produced through animal manure (faeces and urine deposition) and N-fertiliser application. Although most of CH_4 emissions occur through enteric fermentation of the ruminant animals, grassland soils can act as a sink of CH_4. Grassland soil C and GHG emissions has been shown to respond to changes in agricultural management such as fertiliser application, livestock grazing, mowing/cutting in hay meadow systems, and plant composition manipulation, with consequences for soil C stocks (De Deyn et al., 2011, Smith et al., 2008a, Ward et al., 2016).

The processes underpinning soil C and N cycles are sensitive to these global changes, however, the interactive effects of climate warming and management in determining response of above- and below-ground biomass, nutrient cycling and, consequently,

GHG emissions, are poorly understood. In this way, there is an urgent need to better understand these processes to improve predictions of the impacts of climate change on ecosystem functioning and biodiversity (Ostle et al., 2009a). A range of agriculture management strategies have the potential to reduce C losses, increase C sequestration, reduce N_2O emissions and increase CH_4 uptake. This thesis, therefore, focuses on the impact of climate warming and grassland management on the C and N cycling and GHG emissions from soil.

1.2 Carbon and nitrogen cycling in grasslands

1.2.1 Carbon cycling

The C cycle between atmosphere, land and water is essential for sustaining life. Soils have a very high C storage capacity (FAO, 2015), approximately equal to the atmospheric and terrestrial vegetation pools combined (Lal, 2004). This soil C cycle is dependent on the balance between photosynthetic assimilation of CO_2, soil respiration and decomposition of soil organic matter (Trumbore, 2006). Photosynthesising plants, and also photo- and chemoautotrophic microbes, are primarily responsible for transferring C from the atmosphere (as CO_2) to organic pools into the soil (Lu and Conrad, 2005, Trumbore, 2006). These C inputs occurs mainly via plant litter above-ground, root litter, root exudation and through microbial turnover below-ground (Gougoulias et al., 2014). A proportion of the C stored in the soil returns to the atmosphere through root respiration, or via the decomposition of organic material by heterotrophic microorganisms, which is dependent on the C use efficiency (Liang and Balser, 2011). Thus, the production of CO_2 in soils is almost entirely from root respiration and microbial decomposition of organic matter.

Emissions of CO_2 can be lower in waterlogged soils such as rice paddies, peatlands and landfills (Liang and Balser, 2011, Trumbore, 2006) where CH_4 emissions depend on the balance between the production by methanogenic bacteria and the consumption by methanotrophic bacteria (Chistoserdova et al., 2005, Le Mer and Roger, 2001). Methane is produced in anoxic environments by methanogenic bacteria during the anaerobic digestion of soil organic matter, and can be also produced by microbial oxidation in the aerobic zone of methanogenic soils (Le Mer and Roger, 2001). Grassland soils generally act as a sink of CH_4 contributing to a global CH_4 uptake

ranging from 1.32 to 3.03 kg ha^{-1} y^{-1} (Curry, 2007, Yu et al., 2017, Zhuang et al., 2013) mainly due to well-aerated soil conditions (Hartmann et al., 2011, Wang et al., 2014b).

In view of these changes in air temperature and rainfall events, climate conditions have uncertain consequences for grassland C cycling. Temperature and soil water content are important drivers of C cycle processes, mainly affecting microbial respiration and mineralisation rate in the soil, and consequently affecting the release of CO_2 to the atmosphere (Davidson and Janssens, 2006). In addition, temperature and soil water content can affect plant productivity (Olesen and Bindi, 2002), with potential consequences for CO_2 emissions (Wu et al., 2012). These changes in climate are also predicted to increase CH_4 uptake by up to 1.69 g ha^{-1} y^{-1} (Yu et al., 2017), and may affect the net CH_4 flux from soils through altering soil gas diffusivity by changes in soil porosity and water content (Gougoulias et al., 2014, Hartmann et al., 2011). Uncertainties will largely increase due to the interaction between intensified grassland management and climate change.

1.2.2 Nitrogen cycling

Although N is an abundant element in the atmosphere, it is commonly limited in many ecosystems affecting plant growth and productivity as plants can only acquire reactive N forms (Galloway et al., 2014). In grasslands, leguminous plant access N through the biological fixation of atmospheric N_2, while farmers use the addition of manures and fertilisers to increase N inputs. Increases in N availability in soils may, however, result in N losses to the environment indirectly via agricultural activity (volatilisation, leaching and erosion processes), and directly via N_2O production by cycling the reactive N (Butterbach-Bahl et al., 2013). Ammonia (NH_3) volatilisation is highly related to the ammonium (NH_4^+) concentration in the soil, causing acidification and eutrophication of natural ecosystems (Asman et al., 1998) and accounting for up to 42% of farmers surplus N (Burchill et al., 2016). High nitrate (NO_3^-) concentration in soils may lead to greater N leaching losses from soil to water with the potential to cause severe health problems and groundwater contamination (Di and Cameron, 2002).

Production of N_2O is mainly mediated by microbial activities, with two well-studied processes: nitrification and heterotrophic denitrification. Nitrifying bacteria oxidise NH_4^+ or NH_3 to nitrite and nitrate by nitrification. Denitrification by denitrifying

bacteria is the stepwise reduction of NO_3^- to N_2, with an intermediates nitrite, nitric oxide and N_2O (Butterbach-Bahl et al., 2013, Wrage et al., 2001). N_2O is a regular intermediate of denitrification and not nitrification (Wrage et al., 2001). These processes may occur simultaneously in different microsites of the same soil (Stevens et al., 1997), but there is often uncertainty associated with which process are predominantly contributing to N_2O emissions. Recently, researchers have found other processes related to N_2O emissions from soils including nitrifier-denitrification (Wrage et al., 2001) and codenitrification (Spott et al., 2011). The first is related to denitrification by autotrophic nitrifiers and it is a pathway of nitrification. Codenitrification produces a hybrid N_2O (N_2O_{CO}) and N_2 (N_{2CO}) formed from an inorganic N source and another nucleophilic N atom from a co-substrate, but it is rarely studied in soil N processes in the field (Selbie et al., 2015).

The microclimate is recognised to be important for changes in N-processes in the soil. Exponential increases in N_2O emissions with increasing temperature have been reported, with the two main N_2O-formation processes being highly dependent on temperature (Butterbach-Bahl et al., 2013). Changes in soil moisture directly affect oxygen availability to soil microbes, thus controlling the contribution of nitrification and denitrification processes for the production of N_2O (Bateman and Baggs, 2005). Additionally, other factors such as soil C availability and soil pH, are also important drivers for changes in N_2O emissions (Shcherbak et al., 2014). The regulating factors of many N processes remain unclear and further studies are required, especially to identify hotspot emissions from soil mainly given the high spatial and temporal variability. This suggests more work is required to determine the effect of different grassland management strategies and the altered climate conditions.

1.2.3 Relationship between below-ground components and C and N cycling

In the soil, N cycling relies on soil organisms to recycle nutrients for use by plants while C from microbial turnover and metabolism is important for C sequestration (Kallenbach et al., 2016). In grasslands, an important component of the soil microbial community is arbuscular mycorrhiza (AMF) fungi. AMF fungi are obligate symbionts, only obtaining C from the host plant, and can colonise roots of 85% of land plant families (Smith and Read, 2008, Wang and Qiu, 2006). Studies suggested that between 20 and 30% of total C assimilated by plants may be transferred to these fungi (Drigo et al., 2010, Gavito

and Olsson, 2003, Johnson et al., 2002b, Nakano-Hylander and Olsson, 2007), increasing the rhizodeposition (Jones et al., 2004). AMF can also increase the CO_2 uptake with 14% increase in photosynthetic rate in plants associated with AMF (Kaschuk et al., 2010). Another important role of AMF fungi is for soil C sequestration; AMF may improve soil aggregation, providing the C protection below-ground (Rillig and Mummey, 2006). Additionally, AMF fungi can also contribute to soil C by chitin and glomalin production; the last accounts for 30-60% of C in undisturbed soils (Rillig and Mummey, 2006, Treseder and Allen, 2000).

Soil N availability has the potential to alter C concentration in the AMF fungi. When N is available, the host plant limits C translocation to its mycorrhizae, directly reducing C storage in fungal tissues and its residual organic matter (Treseder and Allen, 2000). However, AMF can enhance decomposition of organic matter liberating inorganic N forms to the soil (Hodge et al., 2001), such that AMF fungi may account for 30-50% of N transfer from soil to plants (Govindarajulu et al., 2005, Jin et al., 2005) with the potential to improve plant N nutrition (Blanke et al., 2011, Cavagnaro et al., 2012).

The importance of soil below-ground components, particularly AMF fungi for C and N cycling (Johnson et al., 2006, Veresoglou et al., 2012) and their role in GHG emissions, is currently appears understudied in the literature. Two recent studies have investigated the effect of AMF on CO_2 fluxes (Heinemeyer et al., 2012) and its importance on vegetation at large scale (Vargas et al., 2010). To date, there is no evidence of any study which has evaluated the effect of AMF fungi on CH_4 fluxes, whilst only a few have determined the importance of mycorrhizae fungi on N_2O fluxes (Bender et al., 2015, Cavagnaro et al., 2012). Studies point out that mycorrhizae fungi are potentially a key determinant on soil conditions influencing aggregate stability (Rillig et al., 2002b), pH (Li et al., 1991), nutrient availability (Hodge et al., 2010) and soil moisture (Lazcano et al., 2014). In a forest study, Holz et al. (2016) found that the presence of root and mycelium reduced N_2O emissions and N leaching, despite mineralisation increasing. In addition, it was suggested that an increase of labile C inputs by roots enhance microbial activity and N immobilisation leading to a potential limitation of N_2O production.

One of the challenges to measure GHG emissions from below-ground components is the difficulty to separate fluxes from root and mycelium, which can result in under- or over-estimated fluxes. In-growth core methods have been used to control the presence

of different soil components in the field and to determine the relative contribution of each soil below-ground components to soil respiration (Heinemeyer et al. (2007). Only a few studies have investigated the interactive effect of climate and management on the different components of below-ground respiration (Graham et al., 2014, Heinemeyer et al., 2007). Additionally, to our knowledge, there are no studies which evaluated the presence of root and/or mycelium on CH_4 emissions in grasslands, and only a few evaluated the effect on N_2O emissions (Bender et al., 2015, Bender et al., 2014). Therefore, this thesis presents an in-growth core approach nested within the main field experiment to examine the interactive effects of warming and grassland management on C and N cycling, focusing on the below-ground effects and the consequence for plant/root productivity and GHG emissions.

1.2.4 Plant functional trait and plant composition

Nutrient cycling in grasslands results from a complex interaction between soil microbes, plant traits (leaf and root) and environmental factors. Plant traits relate to aspects of plant function, plant strategies and ecosystem functioning (Abalos et al., 2018, Baxendale et al., 2014, Craine et al., 2001, Lavorel and Garnier, 2002), and may modulate the responses to climate change and grassland management (de Vries et al., 2012).

Ecologists widely use trait-based approaches to characterise plant strategies for nutrient acquisition and plant growth rates. Thus, studies indicate that plant communities dominated by contrasting plant traits may affect soil conditions, which can in turn feedback to influence plant growth (Baxendale et al., 2014). The most commonly studied plant trait trade-off is in relation to nutrient acquisition, specifically fast and slow-growing species. Fast-growing species demonstrate a rapid recycling of nutrients, and thus contain high leaf N content and have higher specific leaf area. These species can be associated with bacterial-based soil food webs and intensively managed grasslands (de Vries et al., 2012). Whereas slow-growing species are related to a slower rates of nutrient cycling, have low leaf N content and lower specific leaf area, and are likely to be associated with more fungal-based food webs and extensively managed grasslands (de Vries et al., 2012, Lavorel and Garnier, 2002, Orwin et al., 2010). Community dynamics and ecosystem processes such as C and N cycling and consequently the production/consumption of GHGs from soil (De Deyn et al., 2008,

Lavorel et al., 2013) might therefore be modulated by the predominant strategy of plant species and their associated plant traits, however this concept has been lacking in the current literature (Abalos et al., 2018).

Furthermore, changes in plant functional composition may alter the communities' root traits, which can influence nutrient cycling via their exudates. Fast-growing species are known to have higher exudate quality, which might promote C and N cycling (De Deyn et al., 2008, van der Krift et al., 2001). Conversely, increases in root exudates may reduce N cycling due to an increase in N immobilisation (Kuzyakov and Bol, 2006). Specific root length (SRL), which is a function of root diameter and root length, is also used for the prediction of nutrient availability and may be different between fast and slow growing species. In particular, SRL usually characterises the economic aspects of the root systems and is often linked to root-nutrient uptake efficiency (Eissenstat, 1992, Eissenstat et al., 2000). In a meta-analysis which summarised the effect of SRL on fertility, soil water content and elevated temperature, Ostonen et al. (2007) suggested that the increase in nutrient availability due to fertilisation, reduced relative root length growth and consequently SRL. It might be possible to correlate this phenomenon to an increase of N_2O production and use SRL as an indicator of potential changes in the release of gases to the atmosphere. Although root traits are less regularly studied than leaf traits, both traits can respond to changes in plant productivity and soil properties, with effects on grassland ecosystem functions (Orwin et al., 2010).

Improved understanding of how plant traits alter community functions may help predict vegetation response to climate change. Soudzilovskaia et al. (2013) found that species with high resources inputs (e.g. thick leaves, low SLA, high C content in roots) increased in abundance at warmer climate allowing their increase for the next season. Additionally, root developmental might change in response to increased temperature, directly, or indirectly by changing in soil moisture (Gray and Brady, 2016). Studies indicate that the root morphology response to increased atmospheric CO_2 concentration (Anderson et al., 2010) and warming (Bjork et al., 2007), are intrinsically linked to root lifespan and turnover. Additionally, Pilon et al. (2013) found out that the changes in root growth rate under different climatic drivers responded differently depending on root diameter size class. Studies, thus, suggest that plant traits may be used to predict below-ground changes due to future climate change, but more work is required.

Management intensification can also change the plant functional composition in grasslands, such as shifting the composition from slow- to fast-growing species. These changes, in turn, can alter soil nutrient cycling and C dynamics (de Vries et al., 2012, Grigulis et al., 2013, Manning et al., 2015). Nevertheless, contradictory effects were found in relation to a relationship between plant traits and soil C; Ward et al. (2016) did not find a relationship between soil C and leaf traits (SLA and leaf dry matter content - LDMC), while Manning et al. (2015) noted a relationship with labile C fractions in the soil surface. Even though studies have advanced the understanding of the role of plant traits on the C and N cycling, and how they may modulate responses to climate change in grasslands with differing management, further studies are needed to appraise potential uncertainties and contribute to finding a consensus (Carrillo et al., 2014).

1.3 Impacts of climate warming on C and N cycling in grassland

Climate change including changes in the soil temperature and moisture could alter C and N cycling and processes in the soil with feedback to plant growth and soil C sequestration thereby affecting rate and direction of GHG exchange with the atmosphere. Studies addressing these changes will improve the understanding of soil C and N and ecosystem function responses to climate change.

In general, most studies have identified that warming may increase soil microbial activities, and consequently the decomposition of soil organic C and mineralisation (Bardgett et al., 2008). In order to ameliorate water stress by warming, there may be increases in C storage below-ground which might increase root productivity and below-ground root biomass (Bai et al., 2010). These changes in C pool processes might modulate the C released to the atmosphere, affecting respiration rates and CO_2 concentration. Wang et al. (2014a) suggested that, on average, warming by 2 °C increased ecosystem respiration by 12%, with the indirect effect of drought offsetting this result. Lu et al. (2013) agreed with this finding and showed that a warming of 1.8 °C increased ecosystem respiration by 6%. These authors also highlighted that soil respiration increased by 9% while its autotrophic and heterotrophic components increased 9.4% and 7.5%. However, these effects are contradictory, with either no effect of warming on ecosystem respiration (Xia et al., 2009) or an adaptation of the ecosystem to warming (Kirschbaum, 2004, Luo et al., 2001, Oechel et al., 2000) being often reported. Chen et al. (2016a) suggested that the contrasting results might be

occurred due to differences in soil respiration components, with above-ground increases relating to autotrophic respiration and microbial biomass C changes relating to heterotrophic respiration. CH_4 uptake might be changed due to increases in temperature, or due to changes in soil moisture. Dijkstra et al. (2013b) observed that warming by 1.5 °C reduced in 15% the cumulative CH_4 uptake in a semi-arid grassland, while Blankinship et al. (2010) showed no effect in another grassland study. The variation in CH_4 uptake can be related to the soil moisture (Dijkstra et al., 2011), affecting oxidation by methanotrophs in the soil and the production by methanogens (Phillips et al., 2001).

Studies suggested that warming might also affect N cycling in grassland soils. Rustad et al. (2001) in a meta-analysis study found that warming increased the net N mineralisation rates by 46%. Similarly in a more recent study, Bai et al. (2013) noticed an increase of 32% of the N mineralisation rate, and an increase of 52% on average net nitrification. Although N mineralised has increased, Bai et al. (2013) found that microbial N immobilisation was not increased by warming, probably because microbes are generally C-limited. A few studies have assessed that warming might not affect N mineralisation (Beier et al., 2008, Niklińska et al., 1999). Variations in the warming effect on nutrient cycling may be due to a result of the interaction of temperature with other abiotic and biotic factors.

The increase of N mineralisation can potentially change the N uptake by plants and/or increase N loss by leaching and/or denitrification as the concentration of NH_4^+ and NO_3^- increases in the soil (Ma et al., 2017). Wu et al. (2012) found out that warming significantly increased above-ground net primary productivity, although it declined over time. It suggests that more rapid N soil turnover, plant N uptake and N loss were associated with a slower decline in the plant productivity response to long-term warming. Ma et al. (2017) observed that warming increased N mineralisation, probably due to an increase of soil microbial biomass and activity. However, soil N availability was not affected evidencing that N could be immobilised by soil microbes or lost through N-leaching or gases N emissions. Turner and Henry (2010) found that warming increased N leaching, mainly due to increases in NO_3^- availability in soils. Additionally, N loss through increases in N_2O emissions may increase exponentially with increase in temperature, while temperature below 10 °C might decrease N_2O formation. Both N_2O-formations processes, nitrification and denitrification, has its optimal temperature for

microbial growth between 20 to 35 °C (Ussiri and Lal, 2012). In contrast, Niboyet et al. (2011) and Zhang et al. (2015b) did not find any warming effect on N_2O emissions in grassland. There are, therefore, many studies, which addressed climate impact in particular warming on C and N cycling. However, studies are not consistent and rarely consider interaction effects with grassland management (cutting and N addition). Studies considering these interactions are required to determine the real effect under a climate change scenario.

1.4 Impact of grassland management strategies on C and N cycling

1.4.1 Nitrogen fertilisation

The application of mineral-N fertiliser in grassland is a standard practice to raise crop productivity and N yield (Suter et al., 2015), but its principles are more complex compared to croplands. According to Ussiri and Lal (2012), N requirements in grassland ecosystems vary throughout the growing season (including changes in the climate conditions); mainly based on changes in species' growth, and due to grazing management and its intensity, influencing grassland C and N cycling.

Nitrogen fertilisation might affect C cycling (Reich et al., 2006), with increases in CO_2 and CH_4 production (Craine et al., 2001, Melillo et al., 2011) and soil C sequestration (van Groenigen et al., 2006). Zhang et al. (2014a) investigating the effects of N addition (9.2 g N m^{-2} y^{-1}) found an increase in CO_2 fluxes through both microbial and root respiration after two years in a grassland ecosystem. Low N application rates (2.3 g m^{-2} y^{-1}) increased only microbial respiration, in particular during the growing season. Likewise, Ambus and Robertson (2006) applying a similar application rate (1-3 g N m^{-2} y^{-1}) over two years did not find an effect on CO_2 flux in grassland. Graham et al. (2014) studying the effect of N application of 5 g N m^{-2} y^{-1} found an increase of 12% in respiration from grassland soil, while Zhu et al. (2016) observed an increase of 19% after similar N application rate (5.6 g N m^{-2}). In contrast, an overdose of 22.4 g N m^{-2} reduced the respiration by approximately 12% from grassland soils (Zhu et al., 2016). As evidenced before, these variations can be potentially related to N addition rate, but also might depend on changes in environmental conditions (soil temperature and moisture) and/or different soil conditions (low pH and/or low C/N ratio) (Ward et al., 2017). The soil C sequestration can also be affected, as Fornara and Tilman (2012)

highlighted with 27 years of N application (10-20 kg N ha^{-1} y^{-1}) to a prairie grassland which promoted an increase of soil C sequestration of 0.11 Mg C ha^{-1} y^{-1} compared to unfertilised grassland, mainly as a result of an increase in root biomass.

Nitrogen addition might also influence the CH_4 uptake in grasslands. Zhang et al. (2017) investigating the effect of N addition in semi-arid grasslands found a reduction of CH_4 uptake by 5.2 ± 0.9 mg C m^{-2} after three years of experimental manipulation. Similarly, Rime and Niklaus (2017) noticed that CH_4 uptake reduced including a reduction in the methanotrophic activity in grassland soils. However, Ambus and Robertson (2006) did not find any effect of N addition on CH_4 oxidation in grasslands. According to Yue et al. (2016), studying the impact of N addition on methane uptake in a five-year experiment, an increase in CH_4 uptake was observed due to increases of N application (0, 1, 3, 9 g N m^{-2}) up to 11.5% in 2011, and then reduced by 2014. The great variability between years was based on the variation of precipitation and temperature, which were shown to be the main drivers of CH_4 flux rather than soil N availability. Yet, other studies suggest that the NH_4^+ concentration in the soil is also highly important due to changes in the microsite affecting CH_4 production in soils (Mosier et al., 1996, Zhao et al., 2017).

As expected, N cycling in grasslands affect N availability in soils. The N transformation rate in the plant-soil system may determine the N availability to plants and the losses of N to the environment. N-losses to the environment may occur via NH_3 volatilisation (Misselbrook et al., 2000), and via N-leaching (Fu et al., 2017). Denitrification as the final step of N cycle, simplified return N_2 to the atmosphere. As previously explained (see Section 1.2.2), nitric oxide and N_2O are produced at intermediate steps before the reduction to N_2. Saggar et al. (2013) found out that globally, temperate grassland can lose 5.6 Tg of N per year via denitrification. According to Cardenas et al. (2010) and Rees et al. (2013), the cumulative N_2O emissions from the UK grazed grasslands range from 0.85 to 51.3 kg N_2O-N ha^{-1}. Additionally, Kim et al. (2013) in a meta-analysis study the majority of studies had a nonlinear relationship (exponential model) between direct N_2O emissions and N-fertiliser inputs. This response can be associated with an excessive N supply beyond plant demands, leading to a lower plant N uptake efficiency, resulting in soil residual N for N_2O production. It is important then to determine the

appropriate N-fertiliser rate to both improve productivity and diminish the N losses to the environment.

1.4.2 Grazing and cutting/mowing in hay meadows systems

Human disturbance associated with agriculture has been recognised as one of the main contributors for changes of the ecosystem diversity and productivity. These disturbances include grazing in uncultivated grasslands (Gillson and Hoffman, 2007) and mowing for hay (Foster et al., 2009). Both grazing and mowing are important grassland management practices, which can modify the C and N cycles by changing the quantity and quality of C inputs to the soil. These changes may greatly affect plant productivity, C allocation, and biodiversity, as well as GHG emissions.

Wang et al. (2016), in a meta-analysis, showed that grazing reduced C stock above-ground by an average of 19% with no changes for shoot N content. Furthermore, grazing may contribute to a reduction of energy allocation to the roots, and root growth enhancing by 5% and 11% of soil C and N pools, respectively, in the top 15 cm soil depth (Wang et al., 2016). Similarly, in a study of the effect of N and mowing in a grassland soil, Wang et al. (2015) also found an increase of 39% in soil total organic C, with no effect on total N, although nitrification rates was increased by 106%.

The limitation of above- and potentially the below-ground biomass due to a reduction of C inputs, may also directly affect soil respiration (Bremer et al., 1998, Curiel yuste et al., 2004). Although many studies has been conducted evaluating the impacts of mowing on soil respiration, the findings are not consistent. Bremer et al. (1998) found that clipping reduced by approximately 18% soil respiration in tallgrass prairie, and Zhou et al. (2007) reported a reduction of about 9% in the clipped plots in grasslands. Contrarily, Antonsen and Olsson (2005) observed higher soil respiration in mowed plots stressing that this could be stimulated by AMF fungi. Han et al. (2012) studying the effect of mowing once a year suggested that the lack of effect on soil respiration in their experiment was due to unaltered soil moisture and plant growth controlling temporal and spatial availability in CO_2 fluxes.

Continued cutting may enhance above and below-ground biomass due to acceleration of plant growth and root exudation (Leriche et al., 2001) and acceleration of the N cycle

(Gusewell et al., 2005) by stimulating microbial mineralisation of soil organic matter and liberating mineral-N (Hamilton and Frank, 2001, Yoshitake et al., 2015). Li et al. (2017a) studying different mowing managements suggested that mowing once a year was the optimal management strategy due to enhanced soil organic matter accumulation, while mowing twice a year should be avoid in a semi-arid grassland. Hamilton and Frank (2001) found that defoliation by grazing promoted an increase plant N uptake due to an increase in the net N mineralisation and soil N availability. However, Zhang et al. (2015b) recorded a non-linear decrease in N_2O emissions with decreases in mowing height. Their results state that a long-term mowing would possibly decrease grassland N_2O emissions from soil. Nonetheless, Gao et al. (2008a) observed an increase of net N mineralisation together with an increase in denitrification, with consequences to N_2O emissions. Likewise, other studies indicated that mowing might affect CH_4 uptake in grasslands. For example Zhang et al. (2012) noticed that mowing increased CH_4 uptake compared to no-mowing plots, with greater increases found during the dry and warm periods in the growing season.

Indirectly, cutting and/or mowing can also alter ecosystem microclimate (soil temperature and soil moisture), increasing evaporation and decreasing transpiration resulting in an unpredictable effect on soil water content (Wan et al., 2002). Such disturbance would in turn influence many of the C and N microbial processes in the soil. Wang et al. (2016) found a reduction of soil moisture (7%) in grazed topsoil, mainly related to the compaction, reducing the infiltration rates through the soil. Additionally, according to Wang et al. (2015), mowing lead to an increase of soil temperature by 0.8 °C over 4 years. Therefore, there are different mechanisms and processes by which mowing may affect C and N cycling in grasslands, and it may vary as a function of vegetation type and soil texture (Han et al., 2012).

1.4.3 Plant composition manipulation - seeding with legumes

Plant-soil interactions have been the focus of many ecological studies due to their effects on global biogeochemical and hydrological cycles in the world (Ostle et al., 2009b). Plant species with different functional groups may modify soil properties, influencing the whole plant community and other ecological processes. The use of plant mixtures with different functional traits might allow the exploration of resources in varied ways (Spehn et al., 2005, Tilman et al., 2001), affecting nutrient cycling and the

plant community productivity. There is a need to understand the plant-soil processes to improve predictions of the impacts on ecosystem functioning and biodiversity, determining potential ways of mitigation (Ostle et al., 2009b).

Manipulation of plant composition in grasslands has mainly been done by seedling of legumes, due to their importance in fixing atmospheric N_2. N_2-fixation by legumes can range from 100 to 380 kg ha^{-1} y^{-1} in northern temperate regions (Carlsson and Huss-Danell, 2003, Ledgard and Steele, 1992), thus improving the N cycling and efficient N utilisation in the whole grassland community, while reducing N inputs via N-fertiliser (Suter et al., 2015). Nyfeler et al. (2009) studying grass-legume mixtures with a legume proportion of about 50 to 70% and plots fertilised with 50 kg N ha^{-1} y^{-1} led to forage yields comparable to grass monocultures fertilised with a rate of 450 kg N ha^{-1} y^{-1}. Further, other studies suggest that soil total N is increased in grass-legume mixtures. Li et al. (2015) found that soil total N and available N are positively related to legume proportion in grass-legume mixtures, but declines when further legumes were added. It is suggested that the increase in legumes promotes an increase of biological N fixation, stimulating nutrient transfer between species (Pirhofer-Walzl et al., 2012). However, further increases in legume proportion might promote an increase of intraspecific competition (Gilad, 2008), limiting the N_2 fixation by legumes species.

Different species composition has been shown to be an important driver enhancing CO_2 uptake and below-ground allocation (Bardgett, 2011, De Deyn et al., 2009), affecting primary productivity and C sequestration (De Deyn et al., 2008). In this sense, De Deyn et al. (2011) highlight that biodiversity restoration practices that align with the promotion of legumes can lead to soil C and N sequestration in a rate of 317 g C m^{-2} y^{-1} and 35 g N m^{-2} y^{-1}, respectively.

Although many studies have addressed the impact of grass-legumes mixtures on the C and N cycling, it is still poorly understood regarding the processes underlining N_2O and CH_4 production and plant species composition (Abdalla et al., 2014, Niklaus et al., 2006, Sun et al., 2013). Niklaus et al. (2006) suggested that high diversity communities lead to a reduction of N_2O emissions due to increasing capture of available mineral-N. Besides, it is highlighted that effects on CH_4 production are changed by high plant composition, and the mechanism underlying is based on the NH_4^+ concentration in the soil. CH_4 oxidation is supposed to be inhibited by NH_4^+ due to substrate competition at

the enzymatic level (Dunfield and Knowles, 1995) or due to complex competitive interactions between nitrifiers and methanotrophs (Powlson et al., 1997). However, it is still unclear the effect of plant manipulation (e.g. different legume proportions) on the GHG emissions, and how the effect would differ with nutrient availability.

1.5 Mathematical modelling approaches

As highlighted in previous sections, measurements of GHG emissions from soil can be particularly difficult due to a range of processes that contribute to the emissions of each gas, and due to the high spatial and temporal variability (e.g. variation in soil temperature and moisture). Besides that, resource limitations can be a considerable barrier to conducting more extensive assessments. To address these issues, mathematical models are used to provide a robust way to estimate GHG emissions, and to interpret the details of the mechanisms behind them. Modelling also gives the opportunity to test different scenarios for various mitigation options (Giltrap et al., 2010). Furthermore, models can be used to predict GHG emissions under different future climate change scenarios, according to IPCC (2000) climate predictions (Abdalla et al., 2014). A range of different models are being used to estimate GHG emissions from soil (e.g. Daycent, ECOSSE, DNDC) requiring basically the same inputs (i.e. soil temperature and moisture content, nutrient availability, soil type, soil pH, vegetation types), but they differ in which variables they take into account.

DNDC (DeNitrification DeComposition) is a dynamic simulation model of C and N biogeochemistry in agro-ecosystems (Fig. 1.1) (Li, 2013). Besides simulating diverse processes of the C and N cycle (Smith et al., 2010), it can also predict crop growth, soil C dynamics, N leaching, and trace gas emissions (Li, 2013). The model was initially developed with three sub-models (climate, decomposition, and denitrification) (Li et al., 1992), and a later version of the model was developed including another sub-model (crop growth) (Li et al., 1994). Finally, in 2000, another two sub-models were added to the model: nitrification and fermentation sub-models (Gilhespy et al., 2014). The soil climate profile is calculated based on the daily climate, soil physical conditions and vegetation inputs, predicting soil temperature and water content. Decomposition sub-model is calculated based on quantity and quality of soil organic C pools, soil climate and soil N availability, calculating C and N pools in the soil. The denitrification sub-model is based on the previous sub-models to estimate the dynamics of dissolved

organic C, N inorganic (NH_4^+, NO_3^-), NO, N_2O. The plant growth is estimated by DNDC based on accumulative temperature, water, N demand, and uptake, simulating the relationships of C and N between plant and soil (Gilhespy et al., 2014). The nitrification sub-model simulates NO and N_2O production via nitrification process, based on nitrification rate and temperature, while the fermentation sub-model simulated the processes related to CH_4 production (Li, 2000, Li et al., 2000). After these daily sequence processes, the DNDC calculates day-by-day until the end of the year, at a site or regional scales.

The DNDC model is well-established and used by a number or research groups across the globe for predictions of GHG emissions with papers published for more than 20 years in, for example, Canada (Smith et al., 2010), across Europe (Abdalla et al., 2009, Kesik et al., 2006), China (Li et al., 2001), and across the world (Giltrap et al., 2010). It has also been widely used in grasslands, showing a reasonable GHG emissions estimation (Brown et al., 2001, Giltrap et al., 2010, Levy et al., 2007, Saggar et al., 2007a), although some modifications are being done to apply to the UK (Brown et al. (2002); UK-DNDC). Effects of grassland management strategies can also be included in the model, e.g. grazing/cutting (Li et al., 2014) and N-fertiliser application (Hsieh et al., 2005), being able to relate this to changes in air temperature and rainfall events. Modelling can then assist experimental researchers by evaluating GHG changes due to a range of interactions between management and climate, determining a real effect over the world. Additionally, it provides the opportunity to estimate emissions inventories due to climate change, and predict for future scenarios.

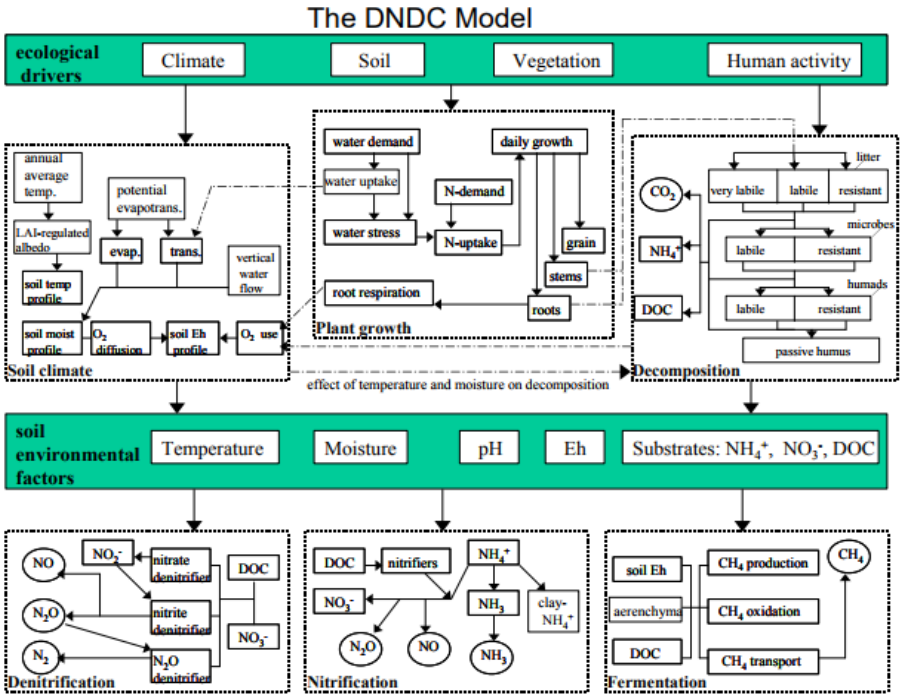

Figure 0.1 DNDC model structure (Li, 2013).

1.6 Thesis aims and objectives

The overarching aim of this research was to investigate and quantify how C and N cycling responds to the interactive effect of climate warming and temperate grassland management, and understand the mechanistic response of plant mixtures with feedback to GHG emissions. Using a field-based experiment, the study aimed to examine the effect of climate warming and grassland management as well as the short-term effect of these interactions on the below-ground response. Additionally, we use a controlled temperature mesocosm experiment to evaluate the mechanistic response of plant composition manipulation on the C and N cycling. Finally, a process-based model DNDC was used to validate the outcomes from the field experiment, the sensitivity analysis of the model, and to predict GHG long-term effect due to the interaction between climate and grassland management. The result could improve our understanding of long-term changes in GHG emission and CO_2 budgets. This thesis comprises three experimental chapters plus one modelling approach chapter. Ultimately, this research addresses the following questions:

How do climate warming and management interact to affect C and N cycling and GHG emissions from temperate grassland soils?

There is increasing concern for grasslands which require a management intensification in order to provide food and fuel for the growing population. At the same time, global warming is expected to increase temperature with further uncertainties for grassland ecosystems. Climate change might then affect C and N cycling in grasslands, but its feedback under different grassland management is still unknown. Improved understanding of the impact of nutrient cycling is important to increase productivity while mitigating GHG emissions and increase soil C sequestration. **Chapter 2** investigates if an interactive effect between climate warming and grassland management affect plant-soil properties, plant productivity, C and N cycling and GHG emissions in a field experiment over two growing seasons. Additionally, this chapter identifies key parameters (plant-root traits, biotic-abiotic) which might explain variations in ecosystem GHG emissions. This chapter has focused on the effect on the ecosystem responses. **Chapter 3** investigates the interactive effect of warming and management on the contribution of soil below-ground components (roots, mycorrhizal fungi, soil microbes) to ecosystem respiration, and specifically how each of the below-ground components might affect C and N cycling and its response to the ecosystem overall. Additionally, the effect of root and/or mycorrhizal fungi in the soil on N_2O and CH_4 emissions are evaluated. Improving this understanding of below-ground C and N cycling is important in grassland ecosystems where evidence is limited.

How does plant manipulation affect C and N cycling and GHG emissions, and does this depend on nutrient availability?

Studies have shown that an increase of plant diversity might improve a range of ecosystem functions, in particular, plant productivity. However, its influence on GHG emissions remains largely unexplored. Additionally, little is known about the effect of changes in plant species proportions in the plant community on nutrient cycling in grasslands. **Chapter 4**, therefore, investigates how different proportions of legumes in grass-legume mixtures affect C and N cycling including GHG emissions, in fertilised and unfertilised soils and examine the potential to act as a mitigation option on a farm scale. Plant-soil interactions govern a range of processes, which a need to be further addressed.

How do future climate change scenarios change GHG balance under an interaction between climate warming and grassland management?

Mathematical models have been shown to reliably simulate GHG fluxes in agroecosystems, allowing the extrapolation of field measurements over different climate scenarios and managements. **Chapter 5**, therefore, simulates GHG emissions under interactions between climate and grassland management using a process-based model (DNDC model) compared to outcomes of field measurements. Additionally, some "what if" scenarios for future climate change and its response in long-term changes in GHG balance are evaluated.

Chapter 6 provides a general discussion of the three experimental chapters. It is a general synthesis discussing the contribution of the work in a broader perspective and the identification of future research needed to improve our understanding how grassland management will influence C and N cycling under future climate change.

2 Interactive effects of climate warming and management on temperate grassland productivity and greenhouse gas emissions

Arlete S. Barneze [1,2], James Heath [1], Jeanette Whitaker [2], Niall P. McNamara [2] and Nick J. Ostle[1]

[1] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

[2] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK.

2.1 Abstract

Globally populations are expected to increase by 2050 meaning that intensification of agricultural land for food and fuel will increase. At this same time global changes including climate warming are likely to impact on terrestrial carbon (C) and nitrogen (N) cycling. Grassland ecosystems will be highly affected, with consequences to soil C sequestration and greenhouse gas (GHG) emissions. Improved understanding of the mechanistic response to these drivers of change, including their interactions, is therefore required and rarely investigated. We used a two-year, full-factorial design field experiment to investigate how climate warming and grassland management interact to affect plant-soil properties, C and N cycling, and GHG emissions from the soil. During the first year, a synergistic interaction occurred; warming increased the N effect increasing ecosystem respiration rates, with no effects on plant productivity. While in the second year, above-ground biomass (AGB) removal interacted with N to reduce ecosystem respiration. Warming decreased the N effect (first year), in an antagonist interaction reducing N_2O fluxes. In addition, AGB removal increased the N effect, increasing N_2O emissions from the soil. Grassland soil was consistently a sink of CH_4; N-only increased the sink by 45% (first year), AGB removal reduced the CH_4 consumption by 44% (second year) as well as warming-only. N availability was an important factor affecting the C fluxes in grasslands. Changes in ecosystem respiration can be explained by microclimate variables, plant productivity and root N content. Soil moisture, specific root length and N pools were responsible for changes in N_2O and CH_4 emissions. Despite single drivers showing greater effects, interactions are crucial to predict for future climate and should be addressed in studies.

Keywords: climate warming, cutting, nitrogen addition, interactions, grassland, C and N cycling, GHG emissions

2.2 Introduction

According to the latest report by the Intergovernmental Panel on Climate Change (IPCC, 2013), the average global surface temperature is likely to rise 1.5-2 °C by the end of the century. Consequently, it is expected that increases in soil temperature and reduced soil moisture (Brzostek et al., 2012) will affect the length of the growing season (Post et al., 2009). These changes may greatly affect carbon (C), and nitrogen (N) cycling and emissions of greenhouse gases (GHGs): carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) from terrestrial ecosystems.

These global changes are particularly important for the sustainability of agricultural ecosystems such as grasslands that support global food security and soil C sequestration (De Deyn et al., 2008, Erb et al., 2016, Garnett, 2009). In order to meet the demand for food by growing populations, sustainable grassland management practices are being intensified to increase yields while reducing environmental impacts (Taube et al., 2014). Intensification through increases in N-fertiliser addition and animal stocking rates are known to affect plant-soil nutrient cycling with feedbacks to productivity and GHG emissions (Garnett, 2009). Global changes including climate warming are also likely to have interactive effects with these intensified grassland production practices with uncertain outcomes. Improved understanding of the mechanistic response to these drivers of change, including their interactions and the potential for synergistic or antagonistic effects, is therefore critical.

The application of mineral-N fertiliser is standard practice in many temperate grassland ecosystems, as it raises crop productivity (Kidd et al., 2017, Lee et al., 2010). However, there are environmental impacts as N additions which can augment N_2O emissions from soil by increasing N availability and stimulating microbial nitrification and denitrification processes (Firestone and Davidson, 1989, Ussiri and Lal, 2012). Many underlying drivers influence these processes, including temperature that affects the regulation of microbial N_2O-formation and N-mineralisation rates (Cantarel et al., 2012). In addition, changes in soil water-filled pore space (WFPS) affects oxygen availability to microbes involved in nutrient cycling and GHG production. Climate

warming can change both soil temperature and soil water content with potential for interactions with N additions. Increases in temperature can accelerate N mineralisation with potential for synergetic interactive effects on plant available N (Rustad et al., 2001), with consequences for N_2O production. The application of N-fertiliser may also alter C cycling increasing CO_2 emissions (Melillo et al., 2011) and/or affect soil C stocks (van Groenigen et al., 2006). However, studies have found large variations in the effects of N addition; with increases or decreases in soil respiration (depending on N addition levels) (Zhu et al., 2016) or no effects (Ambus and Robertson, 2006). The combined effect of N and warming can increase respiration rates due to raised metabolic cycling (Graham et al., 2014). Emissions of CH_4 can also be affected by increases in N availability, changing the CH_4 uptake by soils (Mosier et al., 1996, Zhang et al., 2017). Warming effects on methane are rarely studied, with no effect observed in both multiyear warming experiments (Rustad and Fernandez, 1998, Torn and Harte, 1996), or a decreased in CH_4 uptake early in the growing season observed in a multifactor experiment (Blankinship et al., 2010). Although few studies have examined the influence of warming and N addition (Graham et al., 2014, Jiang et al., 2010), their interaction is far from confirmed and remains uncertain about the consequences of GHG emissions from a grassland soil.

Clipping and its intensity is a critical element of temperate grassland management and function with significant regulatory effects on plant-soil properties and GHG emissions by directly affecting C and N inputs to the soil and net primary production (Fetzel et al., 2017, Petz et al., 2014). Clipping or harvesting the sward has been shown to accelerate plant regrowth and enhance root exudation (Leriche et al., 2001) with impacts on N cycling (Gusewell et al., 2005). This, in turn, can affect soil organic matter dynamics to liberate mineral-N with feedbacks to plant productivity (Hamilton and Frank, 2001, Yoshitake et al., 2015), and N_2O emissions. Clipping will also have an effect on C cycling, decreasing temporarily ecosystem respiration (Bahn et al., 2008). Furthermore, the interactive effect of clipping and N addition may augment even more N_2O emissions due to increase of N available in the soil, and contrary may increase soil respiration due to increase of plant photosynthate production and root exudates trigging soil microbial activity and C cycling (Bahn et al., 2006, Guitian and Bardgett, 2000). The effect of clipping on CH_4 uptake is more related to changes in the microclimate e.g. increasing evaporation and decreasing transpiration, reducing water content in the

soil (Wang et al., 2015). Consequently, lower soil moisture is likely to increase methanotrophy activities, stimulating microbial CH_4 oxidation (Dijkstra et al., 2012). However, clipping interacted to N addition may increase mineral-N availability in the soil, decreasing CH_4 uptake. Studies reflecting the interactive effect between clipping and N addition is scarce in the literature.

There is no evidence of the interactive effect of warming and clipping in grassland soils, although it will depend on the frequency and duration of cutting (Zhou et al., 2007) affecting differently soil components (Bahn et al., 2006), and its effect magnitude on the microclimate (Luo et al., 2001), affecting then nutrient cycling and GHG releases to the atmosphere. Both warming and clipping may also affect nutrient mineralisation, directly affecting the release of GHG emissions. Although single drivers might be important to understand C and N cycling and its feedback on GHG emissions, the interactive effects of these drivers are even more important as it reflects the real world scenario and has been scanting investigated.

Nutrient cycles in grasslands involve complex feedbacks between environmental conditions, plants and soil microbes. There is, however, a lack of experimental studies examining nutrients feedbacks to plant productivity-traits and ecosystem GHG emissions. Microbes and microclimate are well correlated and dominate a variety of processes at the plant-soil-atmosphere interface (Thomson et al., 2013, Zhang et al., 2005), and, plant traits can explain aspects of plant function, plant strategies and ecosystem functioning (Craine et al., 2001, Lavorel and Garnier, 2002).

The challenge for this century is to develop innovative agricultural management approaches, which protect and/or enhance ecosystem biodiversity and functions, including soil C sequestration and the mitigation of GHG, whilst delivering more food sustainably (Ostle et al., 2009b). To address this, most studies have studied only single factors, despite the likelihood that many drivers operate concurrently. Considering the growing expectation for grasslands to supply globally important ecosystem goods, it is important to determine whether the combined effects of multiple factors counteract or strengthen one another as regulators of plant-soil C and N cycling GHG emissions and the consequences for plant productivity.

The aim of this study was to investigate and quantify how climate warming and temperate grassland management, specifically N additions and above-ground biomass (AGB) removal, interacted to affect key plant-soil properties and ecosystem GHG emissions. A second aim was to identify key soil, plant and environmental parameters that explained variation in ecosystem GHG emissions. We predicted that ecosystem perturbations (warming, N addition, AGB removal) would interact to alter plant and soil properties regulating C and N cycling and GHG emissions. Specific hypotheses for this temperate grassland are:

i) N addition and warming interact synergistically to increase plant productivity and GHG emissions, enhancing N_2O and CO_2 fluxes,
ii) AGB removal and N addition interact antagonistically to decrease N_2O emissions and CO_2 release due to C-limitation,
iii) AGB removal and warming interact antagonistically to reduce root productivity and diminish overall GHG fluxes, lowering ecosystem respiration and N_2O fluxes.

2.3 Material and methods

2.3.1 Site description

The experimental site was located at Lancaster University, Lancaster, UK (54º 1'50'' N, 2.7º 46'30''W, 94.1 m a.s.l.) adjacent to Hazelrigg Weather Station (www.lancaster.ac.uk/lec/about-us/facilities/hazelrigg-weather-station). This site is a 61 ha area of permanent unfertilised grassland intermittently grazed by sheep and used as a hay meadow. The vegetation is dominated by grasses including *Holcus lanatus, Anthoxanthum odoratum, Alopecurus pratensis, Poa Trivialis, Agrostis capillaris*, with *Ranunculus repens, Ranunculus acris, and Achillea ptarmisa* also present. The site is under maritime temperate climatic conditions, and the mean annual temperature is 13 °C between 1981-2010 with January being the coldest month (7 °C) and July the warmest (19.5 °C). The mean annual precipitation is 1049 mm (http://www.metoffice.gov.uk). The soil is semi-permeable, seasonally wet, acidic, loamy and clayey according to the National Soil Resources Institute (NSRI), UK soil classification survey (Farewell et al., 2011), and classified as Stagnosols according to FAO classification (FAO, WRB). Initial analyses of the properties of the upper 10 cm

of the soil profile were: total N content 0.3%, total C content 3.5% (inorganic C was negligible), C/N ratio of 12, pH of 5.3 and bulk density of 1.06 g cm^{-3}.

2.3.2 Experimental design

The field experiment used a full-factorial design to test the interactive effects of warming, N addition and above-ground biomass removal totalling eight treatment combinations with five replicates (one within each block). The treatments consisted of: soil-only control, warming-only, N addition-only, above-ground biomass (AGB) removal-only and the interactions N + warming, AGB removal + N, AGB removal + warming, and AGB removal + N + warming. Each block is comprised of 25 plots (3 m^2) in a 5 x 5 grid. For this study, four plots were randomly selected and split to give eight nested treatments (Fig. A1.1 and A1.2, Appendix 1).

The warming treatment was accomplished using an open-top passive conical chamber with an upper opening of 0.66 m, diameter of 1.12 m and a height of 0.40 m based on International Tundra Experiment design (ITEX: Marion et al. (1997), Fig. A1.3, Appendix 1). The transparent material was 2 mm thick polycarbonate sheet (Polycarbonate Shop, Broughton Astley, UK) which allows 92% of the photosynthetically active radiation. The ITEX warming chambers were installed in the field one month prior the beginning of the measurements (April-2015).

Nitrogen addition was applied in May as ammonium nitrate (NH_4NO_3) at a rate of 100 kg N ha^{-1} y^{-1} (consistent with general grassland management recommendation for hay meadows in the UK). For each N addition plot, the fertiliser was dissolved in 5 L deionised water and distributed using a watering can over both plants and soil. No-N plots received an equivalent amount of deionised water only. AGB removal was created manually by cutting and removing the aboveground plant biomass when it reached 5 cm height (i.e. continuous clipping during the growing season).

2.3.3 Greenhouse gases fluxes measurements

A closed static chamber method was used to measure greenhouse gas fluxes (CO_2, N_2O and CH_4) (Ward et al., 2009). A 30 cm diameter, 20 cm high gas sampling base ring was fitted in place to 5 cm soil depth one month prior to the measurements. For each

flux measurement, the chamber was attached to the base ring and 20 mL of chamber air samples were taken via septa using a syringe at 0, 15, 30 and 45 minutes and 10 mL of the chamber air was transferred into a pre-evacuated 3 mL exetainer vial (Labco, Lampeter, UK). The samples were analysed using a PerkinElmer AutoSystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) with a Flame Ionisation Detector (FID) fitted with a methaniser and Electron Capture Detector (ECD) operating at 130 °C. The GC was fitted with a stainless steel Porapak Q 50-80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. Three calibration gas standards (500 ppm, 1000 ppm, 4000 ppm CO_2) (Air Products, Waltham on Thames, UK) were run every 14 samples (Case et al., 2012). Gases fluxes were calculated by fitting linear regressions through sampling time points and were corrected using the temperature and barometric pressure following the ideal gas law (Chadwick et al., 2014, Holland et al., 1999). Microclimate conditions were recorded during each gas sampling date; air and soil temperature were taken using a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, UK) and soil moisture was taken using ML2x Theta Probe and Meter HH2 (Delta T Devices, UK). Greenhouse gas fluxes were measured from the beginning of May 2015 to October 2016. The gases sampling in each year was made immediately after N application in May (approximately 9 a.m.) and then sampling was daily during the first week, then twice a week during the second week followed by every month until October for both experimental years.

2.3.4 Soil sampling and analyses

Three soil cores (Ø = 1 cm, height = 4.5 cm) were taken from each treatment in each plot and kept for the following analysis. Soil samples were taken on days 3, 32 and 72 after N application in 2015 and on days 6, 14 and 63 in 2016 (May, June and July 2015/2016). Days for sampling were determined by rainfall events. Soil gravimetric moisture content was determined after drying at 105 °C for 24 h. Mineral-N (NH_4^+ + NO_3^-) was assessed with 1M KCl in a 1:5 (soil weight: extractant volume) ratio extraction by analysis with a spectrophotometer (Auto Analyser 3 Digital colorimeter BRAN + LUEBBE). Net mineralisation (net NH_4^+ + NO_3^- production) and net nitrification (net NO_3^- production) rate were determined by incubating the soil at 25 °C for 14 days analysing the final mineral-N content as described above, then calculating the daily mineral-N production rate as the difference between final and initial N content,

divided by the incubation period. Soil C and N were determined on dried (60 °C), finely ground soil samples, using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C.

2.3.5 Plant and root sampling and analyses

For the AGB removal treatment (which was continuous clipping during the growing season), plant matter within the GHG measurement chamber was cut and dry matter yield determined by drying at 105 °C for 24 h, on days 8, 22, 42, 72, 120 and 156 (in 2015) and 6, 14, 35, 62, 109 and 155 (in 2016) after N application.

On day 72 and 63 in 2015 and 2016 respectively, all plots were harvested to simulate hay meadow management and plant tissue samples were ground and analysed for total C and N content using an elemental analyser (TruSpec® CN, St. Joseph, MI) at furnace temperature 950 °C. Ethylenediaminetetraacetic acid (EDTA) was used as a reference. Plant traits were also assessed by measuring plant height, leaf dry matter content (LDMC), leaf N content (LNC), leaf C content (LCC) and leaf C/N ratio.

On the day of the final harvest in each year, a soil core (Ø = 5 cm, height = 10 cm) was taken from each plot to determine the below-ground biomass after washing all roots. Before drying the roots at 60 °C to determine the biomass, roots were stored in the fridge with 10% ethanol solution to measure the following root traits: specific root length (SRL), root dry matter content (RDMC), root N content (RNC), root C content (RCC) and root C/N ratio. Root length and diameter were analysed using WinRhizo® root analysis software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) coupled to an Epson flatbed scanner. Root total C and N content were determined on dried root samples, ground and analysed using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C. Ethylenediaminetetraacetic acid (EDTA) was used as a reference.

2.3.6 Statistical analyses

Linear mixed effects models (LME) were used for microclimate data, plant and soil properties and GHG emissions responses to warming, N addition and AGB removal treatments. Fixed effects were warming, N addition and AGB removal and their

interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. For all LME models, data were checked for normality and equal variances using residual plots method and log-transformed where necessary before analysis. Weight functions were used to account for unequal variances following Zuur et al. (2011). The significance of the fixed effects was determined by comparing models with and without the factor of interest using a likelihood ratio test (LRT). All statistical analysis was carried out in the R programming language 3.4.3 (R Development Core Team, 2017) using the additional packages *nlme* (Pinheiro et al., 2017) and *plyr* (Wickham, 2011).

Multiple regression approaches were used to determine the drivers of changes in GHG emissions and the relationship between the plant-soil properties and microclimate variables on GHG emissions. Firstly, data were checked for collinearity using the variance inflation factor (VIF) and scatterplots. Collinear variables were removed from the analysis. Model selection was applied using the remaining variables in both forward and backward selection searching for the lowest AIC (Akaike information criterion). The best-fitted model for each GHG was then checked for normality using a residual plot method. Variables were included in groups and the variation partition was conducted with the R package *vegan* (Oksanen et al., 2017) to determine which groups of variables are most likely to explain CO_2, N_2O and CH_4 emissions.

2.4 Results

2.4.1 Local climate

Climate data from the Hazelrigg weather station showed mean annual air temperature of 9.8 ^oC in 2015 and 10 ^oC in 2016, with soil temperature of 9.4 ^oC and 9.6 ^oC in 2015 and 2016, respectively. Total rainfall was 1332 mm and 1193 mm in 2015 and 2016, respectively. Mean air temperature during the experimental period from May to October was 12.3 ^oC in 2015 and 14 ^oC in 2016 (Fig. 2.1). The hottest recorded air temperature was 23.7 °C in mid-July 2016. The coolest air temperature was 5.8 °C in early May 2015. Mean soil temperature was 14.2 °C in 2015 and 15.7 °C in 2016, an increase of 1.5 °C. In 2016, 569 mm of rain fell in total; 138 mm more rain than 2015. During the month of N addition, 131 and 42 mm of rain fell in 2015 and 2016

respectively. Most rainfall events were less than 5 mm per day and the largest daily rainfall was 50 mm in the end of August 2016 (Fig. 2.1).

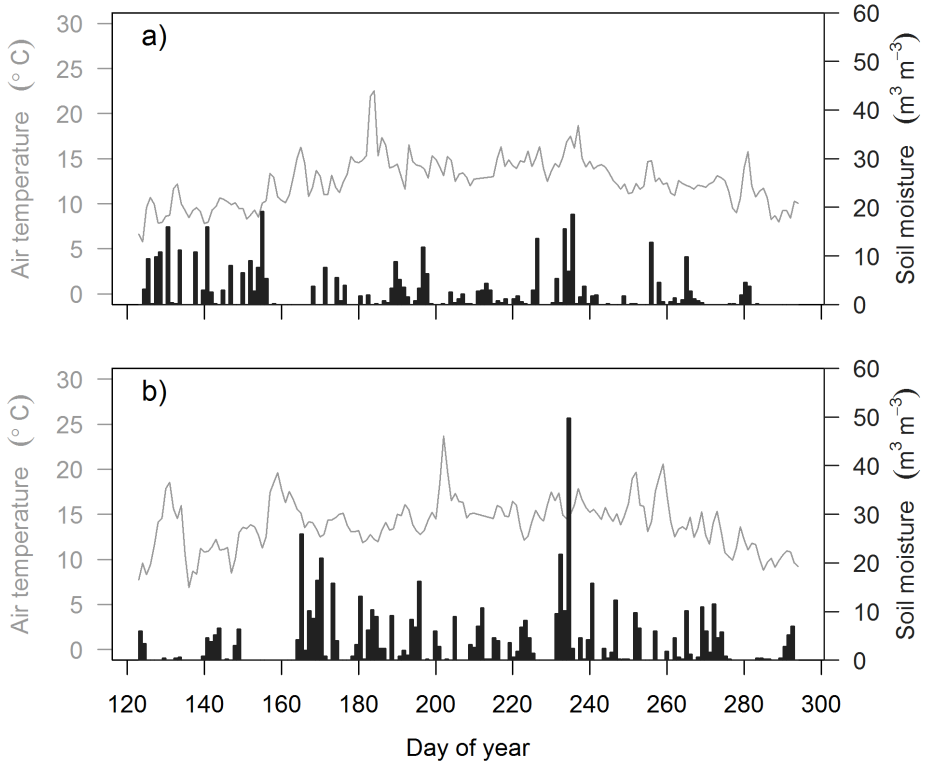

Figure 2.1 Mean daily air temperature (grey lines) and daily rainfall totals (black bars) at Hazelrigg site. Data were collected during the growing season from May to October in a) 2015 and b) 2016.

2.4.2 Seasonal variation in treatment microclimate

Data from the microclimate measurements taken at each sampling time during the experimental period showed the mean air temperature was increased by 2.3 and 2.6 °C in 2015 and 2016 in the warming plots relative to the non-warmed plots (LRT= 58, P<0.0001; LRT= 47, P<0.0001, Table 2.1, Fig. 2.2). Warming also increased soil temperature in both years (4.4% and 2%, respectively), having a greater effect in 2015. Soil temperature was also increased by AGB removal treatment by 2% and 3.7%, respectively for both years; AGB removal left the ground more susceptible to incoming solar radiation. In contrast, nitrogen increased above-ground biomass whilst soil temperature was decreased by 1% and 3.2%, in 2015 and 2016, respectively. Warming had a synergistic interaction with AGB removal, increasing soil temperature in both years. Soil moisture was consistently affected by warming, decreasing by 11% and 17% in both years, respectively (Fig. 2.2). Nitrogen decreased soil moisture, and also interacted with AGB removal increasing soil moisture in 2016.

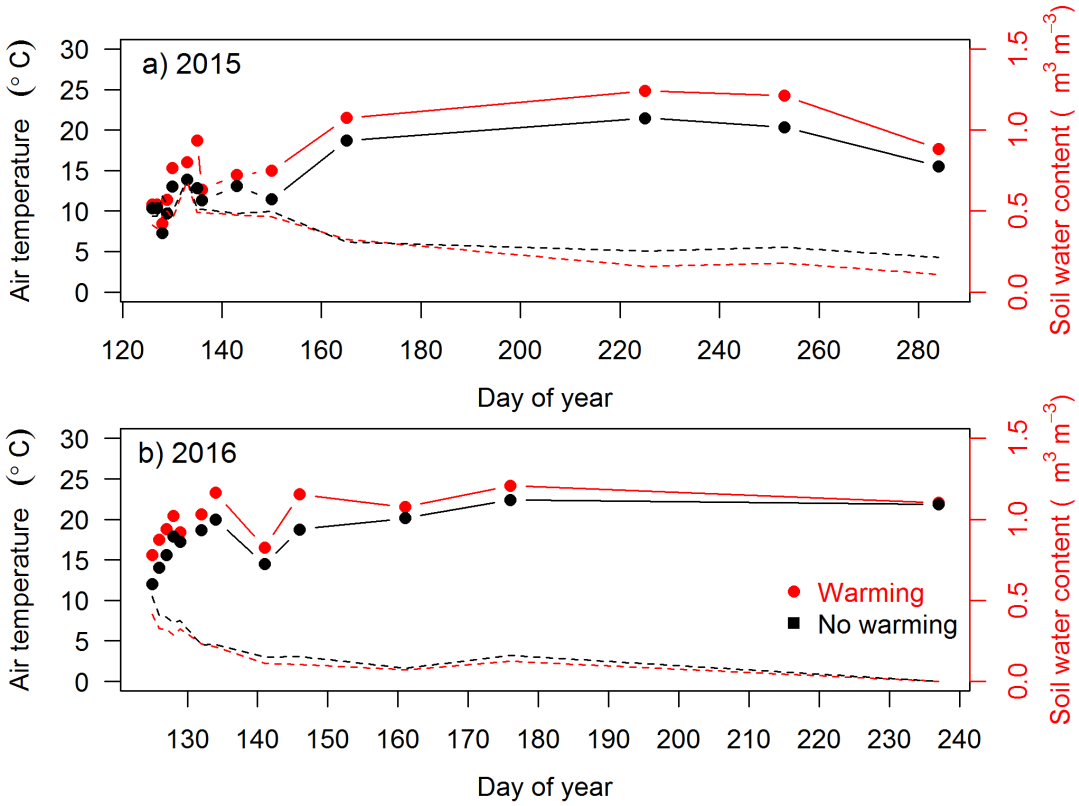

Figure 2.2 Seasonal variation in the warmed and no-warmed plots. Mean air temperature (°C), and soil water content (m^3 m^{-3}), at 100 mm depth in a) 2015 and b) 2016.

Table 2.1 The effect of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on air and soil temperature and soil moisture over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		Air temperature (°C)		Soil temperature (°C)		Soil moisture (%)	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	58.21	**<0.0001**	45.59	**<0.0001**	11.96	**0.0005**
NADD	1	0.06	0.81	5.84	**0.02**	0.01	0.92
AGB REMOVAL	1	1.70	0.19	12.66	**0.0004**	0.64	0.42
WARM *x* NADD	1	0.08	0.77	0.02	0.87	0.11	0.73
AGB REMOVAL *x* NADD	1	0.48	0.49	0.05	0.82	5.55	**0.02**
WARM *x* AGB REMOVAL	1	0.26	0.60	14.42	**0.0001**	1.68	0.19
WARM *x* NADD *x* AGB REMOVAL	1	0.00	0.93	1.17	0.28	4.11	**0.04**
2016		Air temperature (°C)		Soil temperature (°C)		Soil moisture (%)	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	47.52	**<0.0001**	5.09	**0.02**	31.33	**<0.0001**
NADD	1	0.14	0.70	9.35	**0.002**	12.64	**0.0004**
AGB REMOVAL	1	0.17	0.67	26.21	**<0.0001**	3.15	0.08
WARM *x* NADD	1	1.90	0.16	0.215	0.64	1.83	0.18
AGB REMOVAL *x* NADD	1	3.90	0.05	1.48	0.22	11.40	**0.0007**
WARM *x* AGB REMOVAL	1	1.13	0.29	13.17	**0.0003**	0.96	0.33
WARM *x* NADD *x* AGB REMOVAL	1	0.00	0.97	0.40	0.53	4.32	**0.04**

2.4.3 Greenhouse gas emissions

Ecosystem respiration was reduced by 21% and 32% in AGB removal plots in both years (LRT= 28, P<0.0001, LRT= 46, P<0.0001, respectively) (Fig. 2.3, Table 2.2). Nitrogen addition-only increased CO_2 fluxes by 19% and 24% in both years (LRT= 16, P=0.0001, LRT= 24, P<0.0001, respectively) (Fig. 2.3, Table 2.2), with warming-only also increasing fluxes by 9% and 10% each year (LRT= 12, P=0.0004, LRT= 12, P=0.0006, respectively) (Fig. 2.3, Table 2.2). During the first year, a synergistic interaction was observed between warming and N addition whereby CO_2 fluxes increased (LRT= 4, P=0.04). In the second year, AGB removal had a greater antagonistic effect reducing the effect of N on ecosystem respiration rates (LRT= 4, P=0.04) (Fig. 2.3; Table 2.2). Different interactions were observed with N depending on the year.

Nitrogen-only consistently and significantly increased N_2O emissions with the increase being more pronounced in the first year (LRT= 51, P<0.0001, LRT= 19, P<0.0001, respectively) (Fig. 2.4; Table 2.2). AGB removal-only increased N_2O fluxes by 84% and 154% in each year, however, it was not statistically significant (Fig. 2.4; Table 2.2). Warming-only did not affect N_2O fluxes in the first year, while increased fluxes by 30% after two years (LRT= 6, P=0.01) (Fig. 2.4; Table 2.2).

In the first year, warming decreased the effect of nitrogen, with an antagonist interaction reducing N_2O fluxes (LRT= 4, P=0.04) (Fig. 2.4; Table 2.2). In addition, AGB removal synergistically interacted with nitrogen, with a greater increase in N_2O emissions from the soil (LRT= 6, P=0.02) (Fig. 2.4; Table 2.2). After two years, no interaction effects were observed on N_2O emissions.

All treatments were consistent sinks of CH_4. In the first year, nitrogen-only increased the sink by 45% (LRT= 6, P=0.01) (Fig. 2.5; Table 2.2), and after two years AGB removal-only promoted lower consumption of CH_4 by 44% (LRT= 5, P=0.02) (Fig. 2.5; Table 2.2) and warming-only reduced the CH_4 uptake (LRT= 4, P=0.04) (Fig. 2.5; Table 2.2). No interactive effects were found for CH_4 emissions.

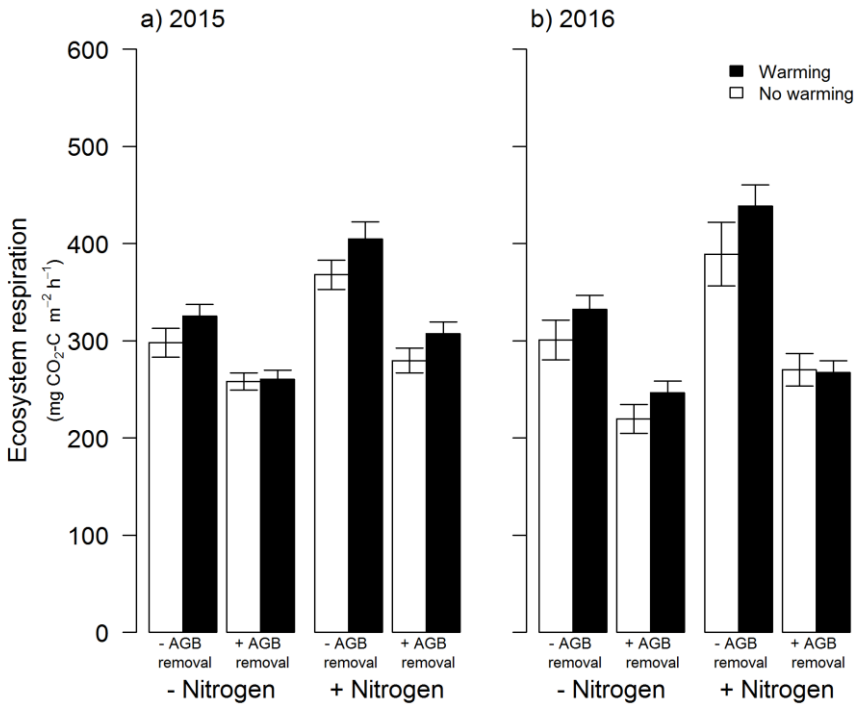

Figure 2.3 Ecosystem respiration in response to warming, nitrogen addition and AGB removal (mg CO_2-C m^{-2} h^{-1}) over (a) 2015 and (b) 2016. Data are mean for all sampling dates ± SE (n=14).

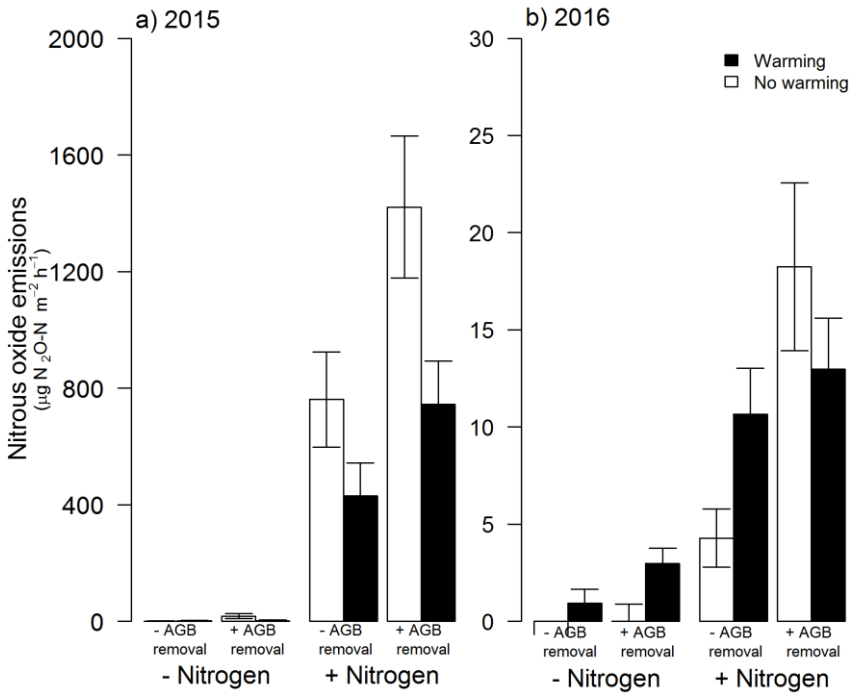

Figure 2.4 Nitrous oxide emissions in response to warming, nitrogen addition and AGB removal (µg N_2O-N m^{-2} h^{-1}) over a) 2015 and b) 2016. Data are mean for all sampling dates ± SE (n=14).

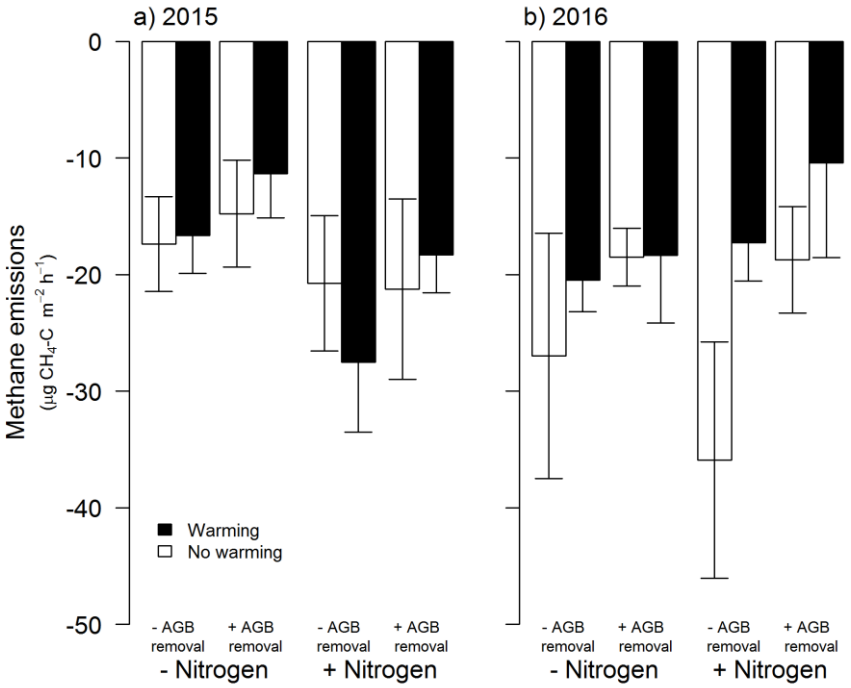

Figure 2.5 Methane emissions in response to warming, nitrogen addition and AGB removal (µg CH_4-C m^{-2} h^{-1}) over a) 2015 and b) 2016. Data are mean for all sampling dates ± SE (n=14).

Table 2.2 The effect of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on CO_2, N_2O and CH_4 fluxes over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		CO_2 emissions mg CO_2-C m^{-2} h^{-1}		N_2O emissions µg N_2O-N m^{-2} h^{-1}		CH_4 emissions µg CH_4-C m^{-2} h^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	12.35	**0.0004**	2.91	0.09	0.94	0.33
NADD	1	16.37	**0.0001**	51.57	**<0.0001**	6.07	**0.01**
AGB REMOVAL	1	28.05	**<0.0001**	2.62	0.10	2.02	0.15
WARM *x* NADD	1	4.18	**0.04**	4.24	**0.04**	0.18	0.67
AGB REMOVAL *x* NADD	1	2.74	0.10	4.70	**0.03**	0.00	0.99
WARM *x* AGB REMOVAL	1	0.02	0.88	1.41	0.23	0.69	0.40
WARM *x* NADD *x* AGB REMOVAL	1	1.02	0.31	0.10	0.74	0.36	0.55
2016		CO_2 emissions mg CO_2-C m^{-2} h^{-1}		N_2O emissions µg N_2O-N m^{-2} h^{-1}		CH_4 emissions µg CH_4-C m^{-2} h^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	11.77	**0.0006**	5.99	**0.01**	4.36	**0.04**
NADD	1	23.97	**<0.0001**	19.09	**<0.0001**	0.02	0.89
AGB REMOVAL	1	46.16	**<0.0001**	3.77	0.05	5.11	**0.02**
WARM *x* NADD	1	0.79	0.37	0.11	0.73	1.70	0.19
AGB REMOVAL *x* NADD	1	4.27	**0.04**	1.41	0.23	0.78	0.37
WARM *x* AGB REMOVAL	1	1.94	0.16	0.01	0.92	1.30	0.25
WARM *x* NADD *x* AGB REMOVAL	1	1.28	0.26	2.19	0.14	0.09	0.76

2.4.4 Plant productivity

As a result of AGB removal treatment, AGB was expectedly reduced by 64% and 68% in both years (LRT= 67, P<0.0001; LRT= 58, P<0.0001, respectively) (Fig. 2.6, Table 2.3). Plant biomass increased 46% and 60% by N addition in both years, (LRT= 22, P<0.0001; LRT= 32, P<0.0001, respectively) (Fig. 2.6, Table 2.3). Warming increased above-ground biomass by 3.7% in the first year (LRT= 10, P=0.001) (Fig. 2.6, Table 2.3), however, no effect was observed in 2016. Below-ground biomass was less influenced by changes in climate warming and management; however, it was observed to increase with AGB removal by 6.3% in 2015, whereas warming decreased below-ground biomass by 16% in 2016 (LRT= 11, P=0.0009; LRT= 11, P=0.001) (Fig. 2.6, Table 2.3).

Root/shoot ratio increased by AGB removal in both years (LRT= 68, P<0.0001, LRT= 74, P<0.0001, respectively) (Fig. 2.6, Table 2.3). Nitrogen decreased root/shoot ratio in both years (LRT= 12, P=0.0006, LRT= 23, P<0.0001) (Fig. 2.6, Table 2.3). Warming had no effect during the first year, while it decreased the root/shoot ratio in the second year (LRT= 9, P=0.002). During the first year, warming and AGB removal interacted antagonistically, decreasing root/shoot ratio (LRT= 5, P=0.03), while AGB removal synergistically interacted with nitrogen (LRT= 5, P=0.02) (Fig. 2.6, Table 2.3).

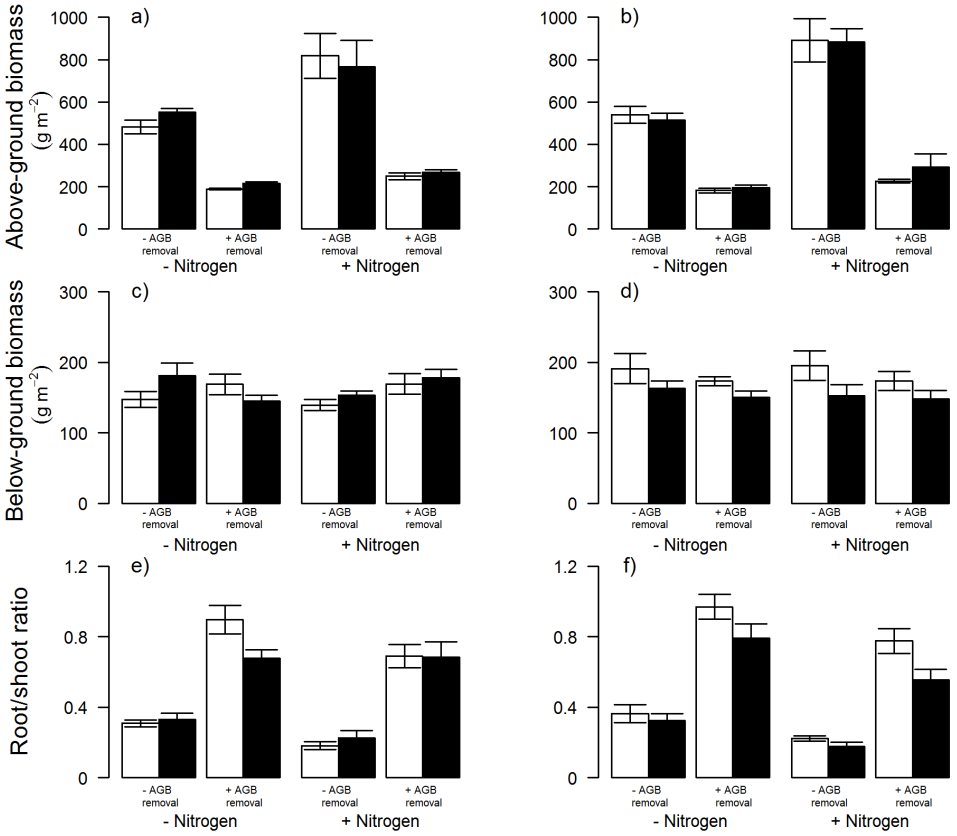

Figure 2.6 Above- and below-ground biomass and root/shoot ratio in response to warming, AGB removal and nitrogen addition over 2015 (a, c and e) and 2016 (b, d and f). White bars show without warming and black bars with warming. Data are mean ± SE (n=5).

Table 2.3 The effect of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on above-ground and below-ground biomass and root/shoot ratio over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		Above-ground biomass g m^{-2}		Below-ground biomass g m^{-2}		Root/shoot ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	10.39	**0.001**	2.88	0.09	0.48	0.49
NADD	1	22.06	**<0.0001**	0.15	0.69	11.89	**0.001**
AGB REMOVAL	1	66.63	**<0.0001**	11.09	**0.001**	67.59	**<0.0001**
WARM *x* NADD	1	1.20	0.27	0.30	0.58	1.88	0.17
AGB REMOVAL *x* NADD	1	1.85	0.17	2.32	0.13	5.34	**0.02**
WARM *x* AGB REMOVAL	1	0.04	0.84	2.16	0.14	4.78	**0.03**
WARM *x* NADD *x* AGB REMOVAL	1	0.76	0.38	3.34	0.07	0.17	0.67
2016		Above-ground biomass g m^{-2}		Below-ground biomass g m^{-2}		Root/shoot ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	0.00	0.93	10.76	**0.001**	9.18	**0.002**
NADD	1	32.46	**<0.0001**	0.12	0.73	23.53	**<0.0001**
AGB REMOVAL	1	58.32	**<0.0001**	1.98	0.16	73.81	**<0.0001**
WARM *x* NADD	1	0.53	0.46	0.15	0.69	0.93	0.33
AGB REMOVAL *x* NADD	1	3.76	0.06	0.03	0.87	2.91	0.09
WARM *x* AGB REMOVAL	1	1.59	0.21	0.18	0.66	0.54	0.46
WARM *x* NADD *x* AGB REMOVAL	1	0.08	0.77	0.18	0.66	0.00	0.94

2.4.5 Plant leaf and root traits

The response of plant leaf and root traits was dependent on climate and grassland management and differed temporally. Warming-only and nitrogen-only treatments decreased LDMC, while AGB removal increased LDMC in both years ($P<0.005$, Fig. 2.7; Table 2.4). After one year, nitrogen antagonistically interacted with warming and AGB removal decreasing LDMC ($P<0.05$, Table 2.4).

RDMC decreased with AGB removal and a synergistic interaction with warming increased RDMC (Fig. 2.8; Table 2.5). No effect was observed in the second experimental year. SRL was influenced differently during the first year by warming; it synergistically interacted with AGB removal increasing SRL and antagonistically interacted with N addition decreasing SRL (Fig. 2.8, Table 2.5). Nitrogen-only decreased SRL in 2016 (Fig. 2.8; Table 2.5).

Leaf and root N content was dependent on N addition and increased in both years ($P<0.05$, Fig. 2.7 and 2.8, Table 2.4 and 2.5). Warming had a synergistic interaction with nitrogen increasing N content in the leaf and root compartments during the first year only ($P<0.05$, Table 2.4, Table 2.5). AGB removal interacted antagonistically with nitrogen decreasing N content in the leaf and root in the first year ($P<0.05$, Table 2.4, Table 2.5). Warming-only had no effect in the first year while it increased leaf N content in the second year. Warming had an antagonistic interaction with AGB removal decreasing leaf N content (Fig. 2.7; Table 2.4).

Root C content was not influenced by any of the treatments. Leaf C content was decreased by warming in the first year, and then decreased by AGB removal and increased by N addition in the second year ($P<0.05$, Fig. 2.8, Table 2.5).

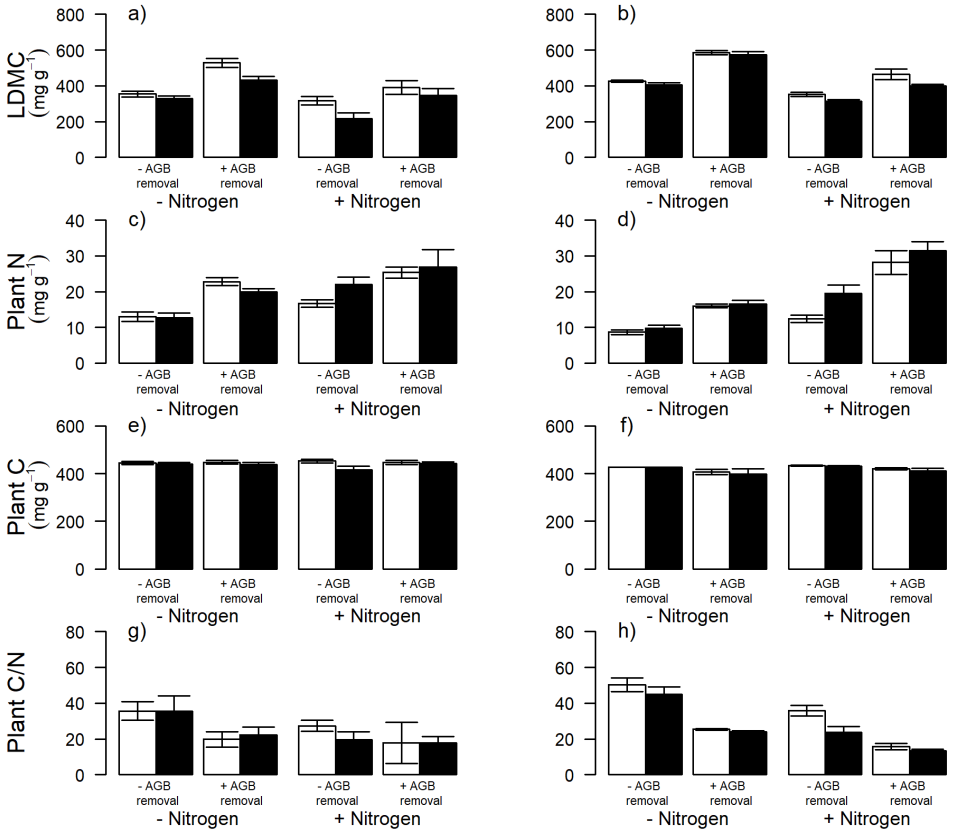

Figure 2.7 Plant leaf traits in response to warming, AGB removal and nitrogen additions over 2015 (a, c, e and g) and 2016 (b, d, f and g). White bars show without warming and black bars with warming Data are mean ± SE (n=5).

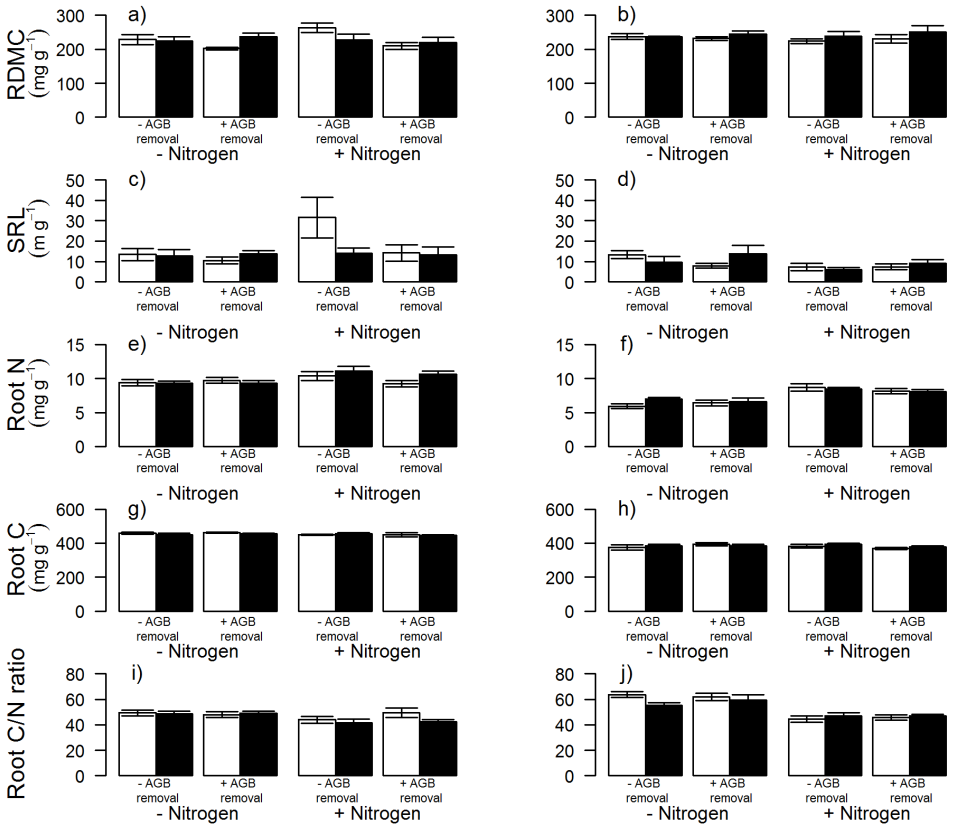

Figure 2.8 Root traits in response to warming, AGB removal and nitrogen addition over 2015 (a, c, e, g and i) and 2016 (b, d, f, h and j). White bars show without warming and black bars with warming. Data are mean ± SE (n=5).

Table 2.4 The effect of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on plant leaf traits over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		LDMC mg g^{-1}		Plant N content mg g^{-1} dry leaf		Plant C content mg g^{-1} dry leaf		Plant C/N ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	13.1	**0.00**	0.8	0.30	4.7	**0.04**	0.2	0.62
NADD	1	16.6	**<0.0001**	19.2	**<0.0001**	0.1	0.78	19.3	**<0.0001**
AGB REMOVAL	1	36.6	**<0.0001**	43.8	**<0.0001**	0.0	0.88	43.0	**<0.0001**
WARM *x* NADD	1	1.3	0.25	5.6	**0.02**	0.0	0.91	7.9	**0.00**
AGB REMOVAL *x* NADD	1	0.0	0.98	6.0	**0.01**	0.4	0.51	8.0	**0.00**
WARM *x* AGB REMOVAL	1	1.0	0.30	4.3	**0.03**	1.7	0.19	5.1	**0.02**
WARM *x* NADD *x* AGB REMOVAL	1	3.8	0.05	0.5	0.46	3.3	0.07	1.7	0.19
2016		LDMC mg g^{-1}		Plant N content mg g^{-1} dry leaf		Plant C content mg g^{-1} dry leaf		Plant C/N ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	8.7	**0.00**	5.3	**0.02**	3.2	0.07	6.9	**0.01**
NADD	1	44.4	**<0.0001**	36.1	**<0.0001**	9.2	**0.00**	42.0	**<0.0001**
AGB REMOVAL	1	79.6	**<0.0001**	57.3	**<0.0001**	10.2	**0.00**	53.0	**<0.0001**
WARM *x* NADD	1	6.3	**0.01**	3.1	0.07	0.3	0.61	1.8	0.18
AGB REMOVAL *x* NADD	1	4.6	**0.03**	0.5	0.49	0.6	0.43	0.2	0.67
WARM *x* AGB REMOVAL	1	0.2	0.63	2.2	0.14	0.6	0.45	1.2	0.27
WARM *x* NADD *x* AGB REMOVAL	1	0.7	0.39	1.2	0.28	0.0	0.87	1.2	0.28

Table 2.5 The effect of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on root traits over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		RDMC mg g^{-1}		SRL m g^{-1}		Root N content mg g^{-1} dry root		Root C content mg g^{-1} dry root		Root C/N ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	0.0	0.88	0.0	0.96	2.5	0.11	0.3	0.56	0.7	0.40
NADD	1	0.4	0.50	3.5	0.06	8.4	**0.00**	2.2	0.14	8.6	**0.00**
AGB REMOVAL	1	3.8	**0.05**	1.5	0.21	1.4	0.24	0.0	0.88	0.2	0.64
WARM *x* NADD	1	2.7	0.10	5.2	**0.02**	6.4	**0.01**	1.6	0.21	3.0	0.08
AGB REMOVAL *x* NADD	1	1.8	0.18	3.0	0.08	3.9	**0.04**	1.5	0.22	1.7	0.19
WARM *x* AGB REMOVAL	1	5.4	**0.02**	4.7	**0.03**	0.2	0.69	0.3	0.59	0.0	0.99
WARM *x* NADD *x* AGB REMOVAL	1	0.0	0.87	0.3	0.6	1.3	0.26	0.7	0.39	1.4	0.22
2016		RDMC mg g^{-1}		SRL m g^{-1}		Root N content mg g^{-1} dry root		Root C content mg g^{-1} dry root		Root C/N ratio	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	2.1	0.14	0.2	0.68	1.1	0.29	0.7	0.41	0.7	0.41
NADD	1	0.1	0.71	7.0	**0.01**	29.7	**<0.0001**	0.3	0.60	39.4	**<0.0001**
AGB REMOVAL	1	0.2	0.65	0.0	0.90	0.6	0.44	0.4	0.53	0.3	0.56
WARM *x* NADD	1	0.8	0.37	0.0	0.83	2.3	0.13	0.6	0.42	5.1	**0.02**
AGB REMOVAL *x* NADD	1	0.4	0.51	1.2	0.27	0.5	0.45	3.2	0.07	0.0	0.90
WARM *x* AGB REMOVAL	1	1.0	0.31	3.4	0.06	0.8	0.36	0.8	0.37	0.4	0.53
WARM *x* NADD *x* AGB REMOVAL	1	0.1	0.76	0.9	0.33	1.4	0.22	0.5	0.49	1.2	0.28

2.4.6 Soil chemical properties

Total soil C content did not change under any of the treatments during over two years ($P>0.05$, Table 2.6). Total soil N and mineral-N were increased by N addition in both years ($P<0.05$, Table 2.6). In the second year, warming decreased soil mineral-N however, this effect was mitigated with N addition. AGB removal increased soil mineral-N ($P<0.05$, Table 2.6). Mineralisation and nitrification rates also increased after N addition in the first year but had no effect in the second year when warming had a positive effect increasing in both years ($P<0.05$, Table 2.6).

Table 2.6 The effects of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on soil chemical properties over 2015 and 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect ($P < 0.05$) are shown in bold.

2015		Soil N g kg^{-1}		Soil C g kg^{-1}		Soil mineral-N mg kg^{-1}		Net Mineralisation rate g kg^{-1} d^{-1}		Net Nitrification rate g kg^{-1} d^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	0.3	0.57	0.3	0.61	0.0	0.96	0.5	0.46	0.3	0.58
NADD	1	5.2	**0.02**	1.3	0.26	56.3	**<0.0001**	5.1	**0.02**	4.3	**0.04**
AGB REMOVAL	1	1.8	0.17	1.4	0.24	1.7	0.19	0.0	0.84	0.1	0.79
WARM *x* NADD	1	0.0	0.94	0.2	0.64	0.0	0.88	0.1	0.70	0.0	0.98
AGB REMOVAL *x* NADD	1	1.1	0.28	0.1	0.79	0.3	0.58	0.0	0.87	0.0	0.97
WARM *x* AGB REMOVAL	1	1.3	0.25	0.2	0.67	2.0	0.15	2.6	0.11	3.0	0.08
WARM *x* NADD *x* AGB REMOVAL	1	0.4	0.48	0.7	0.39	0.04	0.8	0.1	0.72	0.3	0.61
2016		Soil N g kg^{-1}		Soil C g kg^{-1}		Soil mineral-N mg kg^{-1}		Net Mineralisation rate g kg^{-1} d^{-1}		Net Nitrification rate g kg^{-1} d^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*p*	LRT	*P*	LRT	*P*
WARM	1	0.0	0.98	0.0	0.82	4.6	**0.03**	4.6	**0.03**	9.1	**0.00**
NADD	1	9.5	**0.002**	1.0	0.82	69.2	**<0.0001**	0.2	0.62	0.1	0.75
AGB REMOVAL	1	0.8	0.36	0.4	0.54	5.0	**0.03**	0.4	0.54	0.7	0.41
WARM *x* NADD	1	1.0	0.31	0.2	0.64	8.1	**0.00**	2.3	0.13	0.6	0.44
AGB REMOVAL *x* NADD	1	3.4	0.06	0.0	0.87	1.5	0.22	0.7	0.40	0.2	0.63
WARM *x* AGB REMOVAL	1	0.3	0.54	0.2	0.66	1.4	0.24	0.2	0.62	0.4	0.53
WARM *x* NADD *x* AGB REMOVAL	1	0.6	0.41	0.8	0.38	0.02	0.8	0.4	0.53	1.5	0.22

2.4.7 Relationship between plant leaf and root traits, and GHG emissions

Using multiple regression, where all variables were treated independently of the treatment, a model was selected for each of the GHGs using the minimised AIC (Table 2.7). The best model describing ecosystem respiration contained seven variables (AGB, LDMC, root diameter, root N content, air temperature, soil temperature and soil moisture) explaining 77% of the variation found in CO_2 emissions. Using variance partitioning, significant variables were divided into three groups (Above-ground: AGB, LDMC; Below-ground: root diameter, root N content; Environmental: air temperature, soil temperature and soil moisture) with 62% of the variation being be explained by above-ground variables, 4.5% by below-ground variables and 3.8% by environmental variables (Fig. 2.9).

The best model for describing N_2O fluxes contained four variables (soil mineral-N, SRL, soil moisture and N mineralisation rate) and explained 49% of the variation (Table 2.7). Dividing variables into three groups (Soil: soil mineral-N, mineralisation rate; Below-ground: SRL; Environmental: soil moisture) 22% of variation in fluxes can be explained by soil variables, 4.8% by SRL and 5.3% by soil moisture, with 50% remaining unexplained by variables measured in this study (Fig. 2.10).

The best model describing CH_4 fluxes contained six variables (AGB, SRL, soil mineral, RDMC, air temperature and soil moisture) explaining 24% of the variation found in the CH_4 fluxes (Table 2.7). Using variation partition and dividing variables into four groups (Soil: soil mineral; Below-ground variables: SRL, RDMC; Environmental: soil moisture, air temperature; Above-ground: AGB), 15% can be explained by environmental variables, 5.5% by below-ground variables, 3.6% by above-ground and 3.1% by soil mineral-N, with 76% of variation remaining unexplained (Fig. 2.11).

Table 2.7 P-values obtained from multiple linear regressions constructed with significant predictors for CO_2, N_2O and CH_4 emissions. AGB=above-ground biomass; BGB=below-ground biomass; LDMC=leaf dry matter content; LNC=leaf N content; LCC=leaf carbon content; SRL=specific root length; RDMC=root dry matter content; RNC=root nitrogen content; RCC=root carbon content.

Predictor Variables	CO_2	N_2O	CH_4
AGB	8.09e-14	—	0.038
BGB	—	—	—
LDMC	0.026	—	—
LNC	—	—	—
LCC	—	—	—
Root diameter	0.162	—	—
SRL	—	0.006	0.027
RDMC	—	—	0.016
RNC	0.001	—	—
RCC	—	—	—
Soil N	—	—	—
Soil C/N	—	—	—
Soil mineral-N	—	7.28e-06	0.050
Net Mineralisation rate	—	0.001	—
Net Nitrification rate	—	—	—
Air temperature	0.011	—	0.0002
Soil temperature	0.065	—	—
Soil moisture	0.024	0.004	0.0003
Model significance	P < 2.2e-16 Adj R^2 = 0.7758 AIC = 786.606	P=3.099e-11 Adj R^2 = 0.4947 AIC = 1135.95	P=0.0002033 Adj R^2 = 0.2401 AIC = 647.141

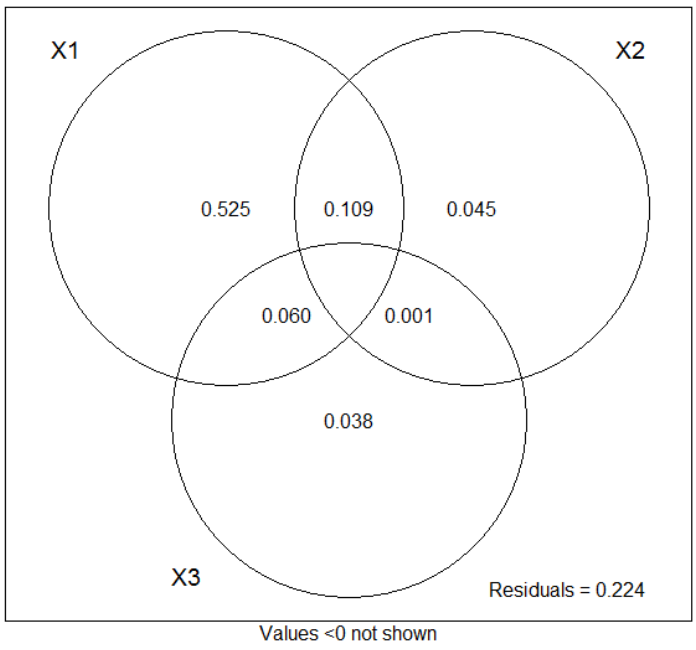

Figure 2.9 Multiple model approach to predict CO_2 fluxes based on plant, root, soil, climate variables. X1 represents above-ground variables (AGB and LDMC); X2 represents below-ground variables (root N content) and X3 represents environmental variables (air and soil temperature and soil moisture). Residuals are variables not measured in this study.

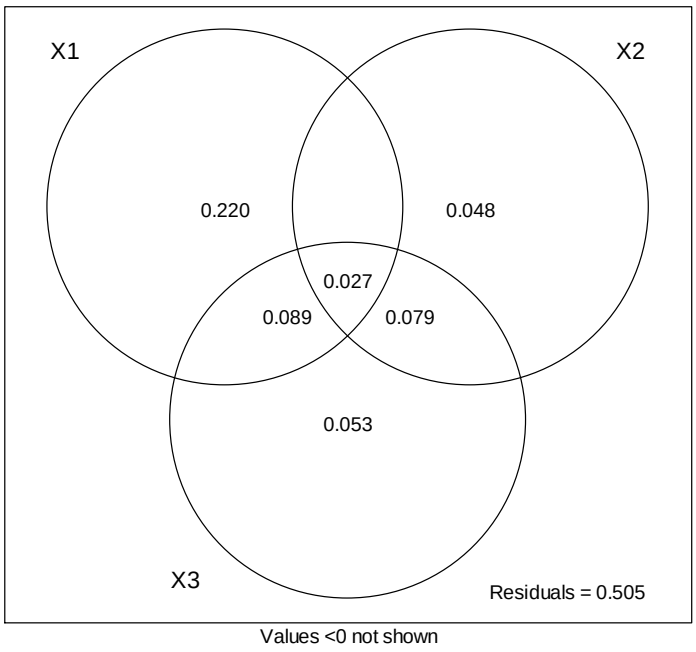

Figure 2.10 Multiple model approach to predict N_2O fluxes based on plant, root, soil, climate variables. X1 represents soil variables (soil mineral-N and net mineralisation rate); X2 represents below-ground variable (SRL) and X3 represents environmental variable (soil moisture). Residuals are variables not measured in this study.

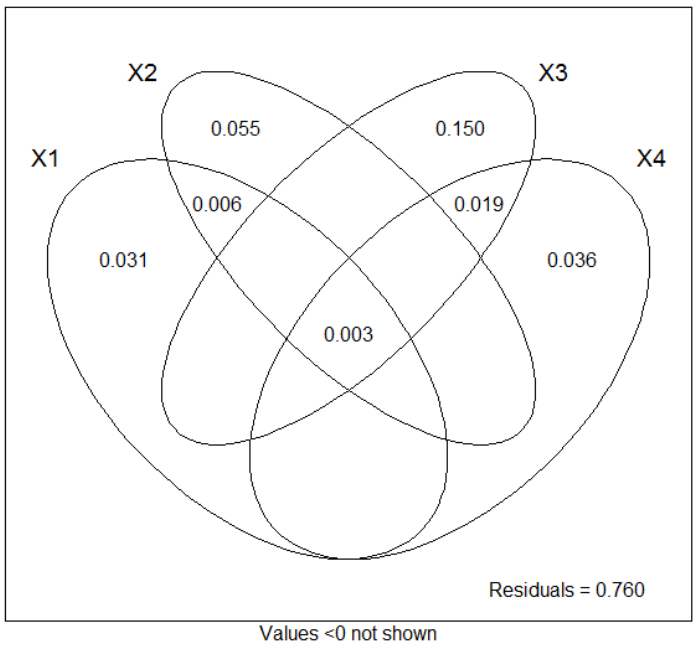

Figure 2.11 Multiple model approach to predict CH_4 fluxes based on plant, root, soil, climate variables. X1 represents soil variables (soil mineral-N); X2 represents below-ground variable (SRL and RDMC), X3 represents environmental variable (soil moisture and air temperature) and X4 represents above-ground variable (AGB). Residuals are variables not measured in this study.

2.5 Discussion

The aim of this study was to investigate how climate warming, N addition and AGB removal interact to affect plant-soil properties with feedback consequences for ecosystem GHG emissions. Above-ground biomass, root traits (such as root N content and SRL), soil mineral-N and air and soil temperature were identified as key drivers of changes in GHG emissions from a grassland soil. Despite the fact that single factors showed greater effects, driver's interactions are crucial and important to predict for future climate world and should be addressed in studies.

Warming increased ecosystem respiration in both study years, although the effect on productivity was only evidenced in the first year. Many factors may be responsible for this, such as soil mineral-N and soil moisture. N_2O and CH_4 emissions were also increased by warming, however only after one year of the experiment. Nitrogen addition as expected raised N_2O emissions, and ecosystem respiration in both years. Methane was only influenced in the first year. AGB removal as expected decreased ecosystem respiration in both years, while N_2O and CH_4 emissions were only influenced after one year of the experiment.

2.5.1 Warming effect

The warming treatment increased air and soil temperature in this study by 2 °C and 0.5 °C, respectively - similar to changes predicted for global air temperature over the next decade (IPCC, 2013). Increasing temperature with open-top chambers may lead to stronger effects due to lower precipitation and extreme temperature spikes (Aronson and McNulty, 2009). Nevertheless, the results found line up well with findings from the literature. As in other studies (Graham et al., 2014, Rustad et al., 2001), climate warming promoted an increase in soil respiration rates of 8% in both years of the experiment. However, increases in above-ground biomass due to increases in ecosystem respiration was only observed in the first year (Fig. 2.6). Warming-induced increases in plant productivity may be directly promoted by higher photosynthesis rates or indirectly affected by increases in nutrient availability (Rustad et al., 2001) or higher soil moisture (Xue et al., 2015). In year 2, soil mineral available N was 50% lower than in year one and soil moisture was 68% lower (in May, after N addition) which may

have been a limiting factor in plant growth (3.8% of CO_2 fluxes variations could be explained by environmental factors, Fig. 2.9).

Warming alone raised N_2O emissions only in the second year, implying that N_2O production from soil is a range of complex processes and may be affected by many factors varying between years (Zhou et al., 2016). Increases in temperature besides increasing N_2O emissions may promote an increase in nutrient supply by mineral-N (Jones et al., 2007, Skiba and Smith, 2000) due to stimulation of nitrifiers and denitrifiers (Tian et al., 2015).

Warming was expected to increase CH_4 uptake (Blankinship et al., 2010, Dijkstra et al., 2013b, Zhu et al., 2015) primarily due to a reduction in soil moisture proving more aerobic conditions to promote CH_4 oxidation (Jones et al., 2005b, Livesley et al., 2009) and higher CH_4 diffusivity (Dijkstra et al., 2012). Conversely, in this study, there was no warming effect in the first year, while a reduction of CH_4 uptake was observed in the second year. Studies suggested that other factors than soil moisture alone may also affect CH_4 emissions including soil texture, soil nutrients, physical diffusion, microbial activity, and the duration of N addition (Carter et al., 2011, Dijkstra et al., 2013b). For instance, increased temperature may cause lower methanotrophic activity, decreasing CH_4 uptake from the soil (Blankinship et al., 2010), which is also showed to decrease the abundance of Type II methanotrophic bacteria in grasslands (Horz et al., 2005). Dijkstra et al. (2012) in a summarised study from field experiments propose that grassland methane emissions are dependent on combined effects of soil temperature and moisture (15% of CH_4 variation is explained by these two variables, Fig. 2.11). Furthermore, a warming effect reduced below-ground biomass (in the second year) probably as part of ecosystem acclimation, which may have reduced soil CH_4 uptake. The below-ground effect can also be related to root traits as SRL and RDMC which accounted for 5.5% of methane emission variation observed in our study, which may be related to changes of root exudation of organic C compounds.

2.5.2 Nitrogen addition effect

In this grassland ecosystem experiment, a positive effect of nitrogen-only addition was observed with increasing respiration rates and enhanced primary productivity in both years (Fig. 2.3; 2.6). Numerous other studies have demonstrated that N addition

stimulates plant growth (Högberg et al., 2006) and plant productivity (Kidd et al., 2017) thus enhancing C inputs to the soil and promoting increased respiration rates (Davidson et al., 2004). The multiple regression also confirmed that plant biomass and LDMC explained most of the ecosystem respiration variations in this study (Fig. 2.9).

Nitrogen addition also promoted a step increase in N_2O emissions in both years, with 2015 being 60% higher than emissions in 2016. The same nitrogen rate was applied in both years, suggesting an ecosystem controlling effect. During the growing season, WFPS in 2015 was higher than 60% while in 2016 it achieved values between 35-40%. N_2O emissions are highly dependent on soil moisture and oxygen availability in the soil (Ussiri and Lal, 2012), suggesting that the main process of N_2O formation (denitrification) was limited in 2016 interrupting mineralisation and nitrification rates (Table 2.6). The greater effect of N addition in the first year could also be due to lack of N application (over 30 years) in the permanent grassland experimental area. Soil mineral-N and soil moisture accounted for 5.3% and 22% of the variation in N_2O emissions after our multiple regression approaches (Fig. 2.10).

Nitrogen addition may also have an influence on CH_4 uptake. Studies suggest that nitrogen addition suppresses CH_4 uptake (Jiang et al., 2010, Zhang et al., 2017), however in this study, during the first year, N increased methane oxidation (Fig. 2.5). During the first year, nitrogen increased NO_3^- concentrations (data not shown) in the soil, which could have contributed to an increase of soil CH_4 uptake as suggested by Jang et al. (2011). Most of the variations in CH_4 fluxes (76%, Fig. 2.11) might be attributed to microbial communities with their activities driving most of methane changes. It is well known that methane dynamics occur due to bacterial methanotrophs and archaeal methanogens, however, unexpected community associations not yet explored might account for greater variations as well (Le Mer and Roger, 2001).

2.5.3 AGB removal effect

AGB removal treatment reduced the above-ground biomass by 21% and 31% in both years, and the ecosystem respiration, therefore significantly reducing C inputs to the soil, limiting substrate availability to microbes (Wan and Luo, 2003). As in other studies (Rafique et al., 2012, Rafique et al., 2011a, Zhu et al., 2015), cutting or grazing-induced increased N_2O emissions in the second year mainly due to changes in N cycling

(Bardgett et al., 1998), accelerating N mineralisation and soil mineral-N (Hamilton and Frank, 2001), consequently augment N_2O emissions from soil.

As suggested by others (Liu et al., 2007, Saggar et al., 2007b), AGB removal treatment decreased CH_4 uptake in the second year which is probably related to the increase in soil mineral-N after cutting, with studies suggesting that raised NH_4^+-N and NO_3^--N concentrations can inhibit soil CH_4 oxidation (Kahkonen et al., 2002, Steinkamp et al., 2001). Ambus and Robertson (2006) suggest a substrate competition theory when methanotrophs compete for NH_4^+ and CH_4 as a substrate. The negative correlation between soil mineral-N and CH_4 can be observed in the multiple model approach, which showed that about 3% of the CH_4 variation could be explained by soil mineral-N (Fig. 2.11).

Accordingly, with our hypothesis, grassland management (N addition and AGB removal) interacted with climate warming; altering soil and plant properties thus regulate C and N cycling and GHG emissions. Below, we will discuss the effect of each interactive factor of grassland management and climate warming.

2.5.4 Interactive effect between N addition and warming

Many studies demonstrate the effects of warming and N addition in diverse ecosystems, however, interactive effects in grasslands are rarely investigated (Graham et al., 2014, Zhu et al., 2015). In partial agreement with our hypothesis, during the first year of the study nitrogen interacted synergistically with warming increasing ecosystem respiration and antagonistically reducing N_2O emissions from soil. No effects on plant productivity were observed.

Warming in a non N-limiting ecosystem was expected to promote greater N_2O emissions due to the acceleration of microbial activity and nutrient cycling (Hoyle and Murphy, 2011) leading to an increase of N mineral transformation in the soil. However, the results showed that warming reduced the effect of N addition, reducing N_2O emissions (Fig. 2.4). This might be related to a reduction of soil water content due to increased temperature (Table 2.1), limiting denitrification processes that favour anaerobic conditions (Ussiri and Lal, 2012). Denitrification is generally considered as the major process driving N_2O emissions from soils (Saggar et al., 2013, Saggar et al.,

2009); however, in this study, the conditions of WFPS lower than 60% may favour nitrification more often than denitrification. Limited denitrification may also be related to lower net nitrification rates in the interaction between warming and N addition, which limited N_2O releases from the soil. Besides denitrification, nitrogen not released may be processed in two ways: i) allocated in the plant leaf and roots, which showed an increased N uptake in warmer conditions (Fig. 2.7, 2.8, Bai et al. (2013)), or ii) increased N uptake by microbes (data not measured). Warming may lead to an increased competition for N, which was limiting in the ecosystem, between plants and microbes (Hodge et al., 2000, Kaye and Hart, 1997), although soil microbes are highly limited by C sources (Bai et al., 2013). Grassland is known to be less affected by warming due to its indirect effect on soil moisture, offsetting temperature effect (Bai et al., 2013). These mechanisms together or separately may be the key factors occurring with a reduction in N_2O emission after N addition in warmer conditions. This could be a climate change positive feedback; nitrogen might be less likely to be released as N_2O.

In agreement with our hypothesis, a synergistic interaction between warming and N addition enhanced ecosystem respiration but no effects were observed on above-ground biomass. In contrast, Gill (2014) did not find an effect on respiration rates after three years of warming and N addition although plant productivity above-ground was increased in a subalpine meadow. The authors suggest that three years was not a sufficient time to change the soil organic matter pools under temperate conditions. In this study, it is possible that N addition increased N uptake by roots (Table 2.5), which requires greater maintenance respiration, since up to half of the root respiration is associated with protein turnover (Scheurwater et al., 2000), enhancing ecosystem respiration and showing no effects on plant productivity. This effect was only observed in the first year, perhaps because soil moisture was reduced in association with N addition in the second year, possibly limiting N diffusion and cycling in the soil.

2.5.5 Interactive effect between N addition and AGB removal

The interactive effect of N addition and AGB removal partially confirmed our hypothesis as ecosystem respiration was antagonistically diminished in the second year, however, N_2O emissions were synergistically raised in the first year, with a consequence for root/shoot ratio which was increased. Zhu et al. (2015) studying the effect of cutting, N-fertilisation and warming in an alpine meadow also found an

increase N_2O emissions in N-fertilised plots interacted to cutting, but it was varied with year. Besides, Wang et al. (2015) observed that mowing and N addition affected ammonification and net N mineralisation rates, which could have an effect on N releases to the atmosphere.

After two years of the experimental treatments, AGB removal reduced the effect of nitrogen on ecosystem respiration (Fig. 2.3) mainly due to the removal of the canopy (Fig. 2.9) which could have increased soil moisture (Table 2.1) (excluding oxygen in the system), promoting a decrease of ecosystem respiration. It is possible that cutting limited N effect on soil microbial communities by reducing labile C sources and root exudation, and reducing microbial activities (Wang et al., 2015). The importance of root N on CO_2 variations over the experimental period accounts for 4.5% according to the multiple regression analyses (Fig. 2.9).

Contrasting from our previous hypotheses, AGB removal interactively increased the effect of N addition during the first year, enhancing N_2O emissions from the soil. Hamilton and Frank (2001) and Yoshitake et al. (2015) suggested that grazing triggers plant growth increasing organic matter input to the soil by plant exudation thereby stimulating microbial mineralisation of soil organic matter and liberating mineral-N, leading to greater N_2O emissions. Cutting stimulates the increase of N in the plant leaf due to rapid use of N to restructure the plant. However, in the presence of N, plant removal decreases plant leaf and root N content (Fig. 2.7; 2.8) promoting a release of N by microbial N_2O production.

Specific root length explained 22% of the variation of N_2O emissions. This suggests that this metric could be used in the future to predict N_2O emissions in grasslands. As SRL usually characterise the economic aspects of the root systems, it is linked to root-nutrient uptake efficiency (Eissenstat, 1992, Eissenstat et al., 2000). Ostonen et al. (2007) and Du et al. (2013) implies that fertiliser application increases nutrient availability and then reduces explorative root growth, decreasing SRL. In this study, higher N_2O emissions showed higher SRL, suggesting that the system had lower nutrient availability due to its release to the atmosphere as N_2O. Almost half of the variation was unaccounted for by our study variables. Some of this unexplained variation might be related to changes in microbial communities of nitrifiers and/or denitrifiers and/or nitrifier denitrifies (Kool et al., 2011, Selbie et al., 2015).

2.5.6 Interactive effect between AGB removal and warming

Contrary to our hypothesis and agreeing with Zhu et al. (2015), there was no interactive effect of AGB removal and warming on GHG emissions overall (Table 2.2), showing that changes in plant (leaf and root) traits and soil chemical properties over the experiment did not influence ecosystem GHG emissions. Differences in plant traits were greater during the first year of the experiment, and can be due to differences in rainfall over the two years (89 mm more in 2015 after N addition), increasing water content in the soil influencing plant-soil properties and C and N cycling (water influence microbial activities and soil properties).

The interactive effect of cutting and warming on GHG emissions will be determined by the balance of i) the rate of above-ground biomass removal and labile C to the soil which will affect ecosystem respiration – soil and microbial respiration (Cao et al., 2004, Raiesi and Asadi, 2006) and N_2O emissions (Dijkstra et al., 2012), and ii) the proportion of increases in soil temperature by cutting (Luo et al. (2010), Dijkstra et al. (2012), Table 2.1).

In terms of plant-soil properties, AGB removal could led to an increase of N available in the soil (Hamilton and Frank, 2001), while warming led to an increase of the N mineralisation (Rustad et al., 2001) leading to a reduction of N availability in the ecosystem (evidenced by the reduction of plant leaf C/N ratio, Fig. 2.7). It also could have affected the root system, increasing SRL (Fig. 2.8) which is known to be increased in N limiting ecosystem, by which roots invest in their structure to acquire N in the soil (Ostonen et al., 2007).

2.6 Conclusions

This study demonstrates that plant productivity and plant-soil properties are strong determinants of GHG feedback emissions. It further concluded that there was an interaction effect between N addition, AGB removal and warming on CO_2, N_2O and CH_4 fluxes although the interactive effect varied among years and background climate condition. Warming interacted with N addition to produce temporal variations in N_2O emissions between years, mainly due to differences in microclimate and increases in N competition. Ecosystem respiration also responded to warming and N interaction enhancing emissions without responses on above-ground biomass. AGB removal

interacted synergistically with N additions, increasing N_2O emissions, and antagonistically reducing ecosystem respiration in the second year. Warming may have resulted in ecosystem adaptation by decreasing below-ground biomass and reducing CH_4 uptake with no effects on above-ground biomass after two years. Plant and root traits offer an alternative means to predict GHG fluxes, mainly N_2O and CH_4 fluxes; however, other studies may be required to access the potential to use traits to estimate ecosystem functional changes. Overall findings show that interactive effects of climate warming and management practices are significant for nutrient cycling in grasslands. Together this means that there is potential to mitigate warming effects with management approaches that improve C sequestration by reducing GHG emissions.

3 Effects of climate and management on grassland soil respiration partitioning

Arlete S. Barneze [1,2], James Heath [1], Jeanette Whitaker [2], Niall P. McNamara [2] and Nick J. Ostle [1]

[1] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

[2] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK.

3.1 Abstract

Grasslands are important ecosystems for the provision of food, fuel and fibre. They represent globally important carbon (C) reservoirs that are under pressure from intensive management and ongoing climate change. How these drivers of change will interact to affect grassland soil C and nitrogen (N) cycling and resultant greenhouse gas (GHG) emissions remain uncertain. The presence of root and/or mycelia in grassland soil is a critical regulator of ecosystem functioning and likely to be an influential determinant of GHG responses to global change. The aim of this study was to investigate the interactive effect of climate warming and grassland management on soil respiration originating from roots rhizosphere, mycelia and free-living microbes. Additionally, we aimed to evaluate the effect of root and/or mycelium on soil C and N cycling and resultant on nitrous oxide (N_2O) and methane (CH_4) fluxes. An in-growth core method was used to partition below-ground respiration. The experiment was conducted in a fully-randomised design to evaluate the interactive effect of warming, N addition, above-ground biomass (AGB) removal and below-ground biotic compartments on GHG emissions and C and N pools in a grassland ecosystem. Results showed that respiration from basal (free-living microorganisms) respiration was highest followed by that from mycelia and roots across all treatments. Warming reduced microbial respiration whilst AGB removal increased it, and CH_4 uptake was reduced by warming overall. N_2O emissions was only affected by N addition, which increased it. Interactive treatments showed an antagonistic interaction between warming and N reducing root respiration, and a three-way interaction between warming, N addition and AGB removal affecting mycelial respiration. The results of this study emphasise the importance of understanding the mechanistic processes where C and N cycling differed

between below-ground components, and how interactions between climate change and grassland management may strengthen or not the effect on soil below-ground respiration.

Keywords: grassland ecosystem, autotrophic and heterotrophic respiration, GHG emissions, N addition, cutting, warming, interactive effects.

3.2 Introduction

Soil contains 4.5 times as much carbon (C) as biotic pools (Lal, 2004, Schlesinger and Andrews, 2000) with biology being influential for this stock (Davidson et al., 2002, Giardina et al., 2003). Specifically, roots and fungal mycorrhizae are important regulators of nutrient cycling and the availability of nitrogen (N) and other elements essential for plant productivity (Johnson et al., 2006, Rillig, 2004). In grasslands, different soil components including plant roots, mycorrhizal associations and soil microbes may also affect nutrient cycling with feedback to greenhouse gas (GHG) emissions (Dijkstra et al., 2013a, Paterson, 2003, van Groenigen et al., 2015) and C storage below-ground (Büscher et al., 2012, Johnson et al., 2006). However, studies on the contribution of specific below-ground biotic components to grassland soil respiration and C and N cycling, in particular, the contribution of mycorrhizae fungi is limited (Heinemeyer et al., 2012, Johnson et al., 2002a) and rarely consider the effects of interactions between climate change and management. Studies have considered only one driver of change (e.g. temperature/moisture; Heinemeyer et al. (2007), liming; Johnson et al. (2002a)) which does not translate the real world scenario.

In general, intensively managed grasslands, such as by N fertilisation and mowing/grazing can alter temperate grassland ecosystems by altering e.g. plant productivity and nutrient cycling (Giese et al., 2013, McSherry and Ritchie, 2013). These effects might affect directly the below-ground C and N storage, by changing below-ground biomass and net primary productivity (Bai et al., 2015, Gao et al., 2011, Gao et al., 2008b). Climate warming might further emphasise these effects, with consequences, in particular, for C and N cycling and different GHG emissions from the below-ground component. In view of this, it is crucial to understand how these below-ground compartment will be affected by interaction between management and climate change for the grassland sustainability.

Heterotrophic respiration results from soil organic matter decomposition by microbes while autotrophic respiration is related to the respiratory activity of roots and associated microbes. The former primarily controls soil C storage and nutrient dynamics whereas the latter reflects plant activity and the supply of organic compounds to roots from the plant canopy (Hanson et al., 2000). Soil respiration is derived from roots, fungal mycelium (mostly found as arbuscular mycorrhizal (AMF) fungi in grasslands, Johnson et al. (1992)) and free-living microorganisms. There is a large uncertainty associated with the amount of respiration originating from each of these sources due to variations in ecosystem properties and the interactive effects of climate change and management/land use (Jones et al., 2009, Nguyen, 2003). There are some studies which evaluate the partitioned soil below-ground respiration into roots, mycorrhizae and microbes (e.g. in forest: Heinemeyer et al. (2007) , in barley field; Moyano et al. (2007)), but there are only a few to date in grasslands ecosystems due to difficulty in separating root from mycorrhiza hyphal respiration. Heinemeyer et al. (2012) in a grassland study found that mycelium respiration contributed to 27% of soil respiration, while root respiration contributed to 11% however, they varied considerable across the experimental period. Considering mycorrhizae and root together, Graham et al. (2014) and Zhang et al. (2014a) both found that heterotrophic respiration formed the largest contribution to grassland overall soil respiration and this was due to microbial communities being able to access both old (C resident in the soil for decades, Trumbore (2000)) and recent soil C (Dijkstra et al., 2013b). Yet the assessment of each below-ground component (in particular mycorrhizae and roots) as distinct sources is required given that they may respond somewhat independently to environmental changes (Alberton et al., 2005).

Whilst relatively under researched soil nitrous oxide (N_2O) and methane (CH_4) fluxes are also likely to be affected by the presence of roots and/or mycelium. Roots and/or mycelium may have an indirect effect on N_2O fluxes through alteration of soil nutrient availability, affecting plant growth and nutrient acquisition (Bender et al., 2015, Cavagnaro et al., 2012), and CH_4 uptake by changes soil conditions and microclimate (Hodge et al., 2010, Rillig and Mummey, 2006). Thus, it is important to evaluate the key role of each soil below-ground compartment on the C and N cycling.

Increases in temperature, due to climate change, may affect both autotrophic and heterotrophic soil respiration, affecting, directly and indirectly, ecosystem respiration (Chen et al., 2016a, Graham et al., 2014, Peng et al., 2015). Directly, warming can affect mycorrhiza and its colonisation (Heinemeyer and Fitter, 2004), and indirectly the C allocation from the host plant (Heinemeyer et al., 2006, Rillig et al., 2002b). According to Smith and Read (2008), autotrophic respiration is highly dependent on substrate supply from photosynthesis; therefore, it will influence C inputs and cycling in the soil. Additionally, warming is expected to accelerate nutrient mineralisation, and fine root growth could decrease due to less C needing to be allocated to below-ground under increased N availability (Dieleman et al., 2012). Heterotrophic respiration, in turn, can be affected by warming due to an increase in microbial biomass (Lu et al., 2013) and decomposition of stable organic matter (Hopkins et al., 2012).

As part of intensified grassland management N-fertiliser addition, for increasing biomass productivity, can effect soil respiration and its component contributions, however studies show a great variability and a lack of consistent results (Tu et al., 2013, Zhang et al., 2014b, Zhou et al., 2014). The application of N-fertiliser may increase soil microbial biomass and activity (Allison et al., 2010) or decrease enzyme activity and soil organic matter decomposability (Treseder, 2008), resulting in corresponding changes in heterotrophic respiration. Likewise, N addition may stimulate autotrophic respiration due to increased plant growth and root biomass (Cleveland and Townsend, 2006) or suppress it by reducing below-ground C allocation (Giardina et al., 2004, Kuzyakov, 2002, Wang et al., 2017b) resulting in changes in autotrophic respiration. Additionally, increases in cutting/grazing or harvesting for biomass has been shown to negatively affect soil respiration and its components (Bremer et al., 1998, Wei et al., 2016) especially in the short term (Bahn et al., 2006). Autotrophic respiration might be less affected due to existing carbohydrate reserves which sustain root metabolism whilst heterotrophic respiration strongly responded negatively to short-term changes in assimilate supply (Bahn et al., 2006). Grazing is also suggested to affect mycorrhizae fungi due to C limitation (Heyde et al., 2017, Sonnemann et al., 2016) by the reduction of above-ground biomass (AGB).

Although studies of the effects of single drivers (abiotic or biotic) on soil functions are valuable, ecological drivers occur simultaneously in the real world interacting in ways

that may be difficult to predict and rarely tested experimentally. For example, studies have shown that interactions between warming and N showed no effect on the partitioned respiration in a grassland ecosystem (Graham et al., 2014) whereas changes in nitrification processes were recorded in a forest ecosystem (Liu et al., 2011b). In other grassland experiments warming interacted with AGB removal did not result in significant changes in partitioned soil respiration (Zhou et al., 2007), whereas Zong et al. (2017) discovered that N and cutting increased below-ground biomass but did not affect the partitioning of soil respiration.

A range of methods have been used to partition soil respiration, and these include the use of stable isotopes, trenching to remove roots, and in-growth cores (Hanson et al., 2000, Johnson et al., 2001, Trumbore et al., 2006). There is still no generally accepted method as all have caveats leading to over and underestimations of soil respiration (Neil, 1992). In-growth mesh-cores are often used because of their simplicity of design (Chen et al., 2016b, Milchunas, 2009), allowing the free movement of water, bacteria and nutrients through the mesh (Moyano et al., 2007). These cores also have the advantage of enabling the separation of soil respiration from the root and mycorrhiza fungi soil components (Heinemeyer et al., 2007, Johnson et al., 2001).

Given that the ecosystem respiration on the ecosystem level (Chapter 2) was significantly altered by treatments, but the response on below-ground biomass was less clear, the aim of this study was to investigate the interactive effects of warming, N addition and AGB removal on the partitioned grassland soil respiration derived from roots, mycelia and free-living microbes in the field. Additionally, we evaluated the effect of roots and mycelia on soil C and N cycling and resultant N_2O and CH_4 fluxes.

As mycorrhizal respiration is normally accounted together with root respiration, we hypothesised that in general, it represents a substantial component of below-ground respiration, which needs to be considered. Respiration from root and mycorrhizae might respond differently either to abiotic or biotic ecosystem changes. Additionally, i) warming or N addition will increase both autotrophic (root and mycelia) and heterotrophic respiration and productivity due to increase in root biomass, ii) while cutting will affect negatively heterotrophic respiration with no response on the autotrophic respiration, due to their influence on C storage below-ground. Whereas interactive effects may change the direction of the effects on soil respiration: a)

warming and N addition will interact synergistically, increasing both autotrophic and heterotrophic respiration and plant productivity, due to an increase in root biomass, b) a synergistic interaction between warming and AGB removal will increase both autotrophic and heterotrophic respiration mainly due to the effect of warming on the root biomass and the effect of C limitation for soil microbes; and c) N addition will synergistically interact to AGB removal, increasing both heterotrophic and autotrophic respiration, due to increase N availability in the soil.

To test these hypotheses, a field study was conducted using in-growth cores nested within the individual and interactive warming, N addition and AGB removal treatments of the main experiment (described fully in Chapter 2). In each treatment, three soil in-growth manipulation cores were inserted to either exclude roots and hyphae or allow ingress of roots and hyphae, and hyphae only. Soil respiration was thus partitioned between each below-ground component, and N_2O and CH_4 emissions were also measured. Soil properties and nutrient availability were evaluated to determine the effect on C and N cycling in a grassland ecosystem.

3.3 Material and Methods

3.3.1 Site Description

The experimental site was located at Lancaster University, Lancaster, UK (54º 1’50’’N, 2.7º 46’30’’W, 94.1 m a.s.l.) adjacent to Hazelrigg Weather Station. This site is a 61 ha area of permanent unfertilised grassland which is owned and managed by Lancaster University and has been intermittently grazed by sheep and used as a hay meadow. The site is under maritime temperate climatic conditions, and the mean annual air temperature was 13 °C between 1981-2010 with January being the coldest month (average of 7 °C) and July the warmest (average of 19 °C). The mean annual precipitation is 1049 mm. The soil is semi-permeable, seasonally wet, acidic, loamy and clayey according to the National Soil Resources Institute, UK soil classification survey (Farewell et al., 2011), and classified as Stagnosols according to FAO classification (FAO, WRB). Initial analyses of the properties of the upper 10 cm of the soil profile are total N content 0.3%, total C content 3.5% (inorganic C was negligible), C/N ratio of 12, pH of 5.3 and bulk density of 1.06 g cm^{-3}.

3.3.2 Experimental in-growth core design

The in-growth core experiment was nested within the main experiment (Chapter 2, Section 2.2.2) totalling eight treatment combinations with five replicates (one within each experimental block). The treatments were soil-only control, warming, N addition and AGB removal, and the interactions AGB removal + warming, N addition + warming, AGB removal + N addition and AGB removal + N addition + warming. In each main treatment, three soil in-growth manipulation cores were inserted to allow either ingress of roots and hyphae, hyphae only or no hyphal or root inputs.

The soil in-growth cores were made based on the design of Johnson et al. (2001), which consists of plastic drainage pipe (6.8 cm diameter and 15 cm depth) with two slots (5 and 10 cm) cut into the sides to adhere exclusion mesh for the given in-growth treatments. For excluding roots or mycelium, or both, nylon closed-bottom mesh bags (Plastok Associates Ltd, Birkenhead, Wirral, UK) were adhered to the pipe (Fig. A2.1, Appendix 2). The three types of in-growth cores (40 of each type) were filled with approximately 500 g of fresh (soil moisture approx. 49%), sieved (through 4 mm mesh) and root-free soil taken from within each main treatment (120 in-growth cores in total). Root/mycelia cores allowed root and mycelial in-growth using a 2 mm mesh; mycelia cores excluded roots but allowed mycelial in-growth (35 µm mesh) and no in-growth was achieved through using 1 µm mesh (Fig. A2.2, Appendix 2). One core of each mesh size was inserted in each plot to 15 cm depth in the soil and nylon top-covered mesh. The cores were installed in May 2015 and allowed to settle for one year before GHG measurements were made during May and June 2016.

3.3.3 Soil respiration and GHG flux measurements

Measurements of soil respiration, N_2O and CH_4 fluxes were made on each root in-growth cores in May and June 2016. GHG sampling lids were made using drainage pipe (6.8 cm diameter and 9 cm depth, Screwfix, UK) fitted with a lid and septum for gas sampling (Fig. A2.3, Appendix 2). For each flux measurement, the lid was secured to the in-growth core and 5 mL gas samples (t_0) were taken immediately and then after 30 minutes (t_30) using a 10 mL syringe. Gas samples were transferred to 3 mL pre-evacuated exetainer vials (Labco, Lampeter, UK) for storage until analyses. Gas samples were analysed using a PerkinElmer Autosystem XL Gas Chromatograph (GC)

(PerkinElmer, Waltham, MA, USA) with a Flame Ionisation Detector (FID) fitted with a methaniser and Electron Capture Detector (ECD) operating at 130 °C. The GC was fitted with a stainless steel Porapak Q 50-80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. Three calibration gas standards (500 ppm, 1000 ppm, 4000 ppm CO_2) (Air Products, Waltham on Thames, UK) were run every 14 samples (Case et al., 2012). Gas fluxes were calculated by the difference in (t_0) and (t_30) gas concentrations corrected for air temperature and barometric pressure following the ideal gas law (Holland et al., 1999). Air and soil temperature were taken using a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, UK) and soil moisture was taken using ML2x Theta Probe (Delta T Devices, UK) at each gas sampling date inside each main treatment (and outside the exclusion cores). Following N additions in May 2016, CO_2, N_2O and CH_4 measurements were made between 3rd June and 23rd June, totalling 11 sampling occasions.

3.3.4 Soil and root analyses

Soil cores were removed from each plot for physical and chemical analyses at the end of the GHG measurement period on the 24th June 2016. Soil gravimetric moisture content was determined after drying 5 g of soil at 105 °C for 24 h. Soil bulk density and water-filled pore space (WFPS) was calculated. Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted with 1M KCl in a 1:5 (soil weight: extractant volume) and analysed with a spectrophotometer (AutoAnalyser 3 Digital colorimeter BRAN + LUEBBE). Soil C and N were determined on dried (60 °C), finely ground soil samples, using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C. From the remaining soil, the below-ground biomass was determined after washing all root and dried at 60 °C.

3.3.5 Calculations and statistical analyses

Linear mixed effects models (LME) were used to test for treatment effects on soil properties and GHG emissions (i.e. to account for the overall effect of the main treatments and in-growth cores, n=11) to the responses to warming, N addition, AGB removal, and in-growth cores. Fixed effects were warming, N addition, AGB removal, and in-growth cores and their interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. For all LME models,

data were checked for normality and equal variances using residual plots method and log-transformed where necessary before statistical analysis. Weight functions were used to account for unequal variances following Zuur et al. (2011). The significance of the fixed effects was determined by comparing models with and without the factor of interest using a likelihood ratio test (LRT). Being the fixed term "partitioned below-ground PBP" significantly different, Tukey post-hoc analyses were carried out and a significant effect was determined at $P \leq 0.05$. All statistical analyses and graphs made using R 3.4.3 (R Development Core Team, 2017) using the additional packages *nlme* (Pinheiro et al., 2017) and *plyr* (Wickham, 2011).

To account for the effect of different below-ground components on ecosystem respiration rates, the absolute contribution of fine roots, mycelial and free-living soil microbes was calculated and partitioned according to Moyano et al. (2007):

i.e.

Total below-ground respiration flux = 2 mm mesh cores

Root rhizosphere respiration = (2 mm – 35 µm)

Mycelial respiration = (35 µm – control)

Basal respiration = control (1 µm mesh core)

Partitioned ecosystem respiration was analysed as described above. The output showed that below-ground components were different and statistical analyses were made for each below-ground component. LME were used for each partitioned ecosystem respiration and microclimate data response to warming, N addition and AGB removal. Fixed effects were warming, N addition, AGB removal, and their interactions. The random effect was split-plot nested within block to take account of the experimental split-plot design. For all LME models, data were checked for normality and equal variances using residual plots method and log-transformed where necessary before analysis. Weight functions were used to account for unequal variances following Zuur et al. (2011). The significance of the fixed effects was determined by comparing models with and without the factor of interest using a LRT.

3.4 Results

3.4.1 Treatment microclimate

During the experimental period, the absolute maximum air temperature observed was 29 °C with a minimum of 8 °C, and an absolute maximum soil temperature of 23 °C and minimum of 1 °C. Microclimate measurements were taken at each main treatment plot to coincide with the 11 GHG sampling occasions in May and June 2016. From this period, results showed that mean air temperature was raised by 2.5 °C (LRT=168, P<0.0001, Table 3.1), and soil water content was reduced by 18% (LRT=47, P<0.0001, Table 3.1) in the warmed plots relative to the non-warmed plots (Fig. 3.1). Mean soil temperature was reduced by N-fertiliser application (LRT=7, P=0.01, Table 3.1), and increased by AGB removal (LRT=10, P=0.001, Table 3.1). Interactive effects showed that N affected soil moisture in the AGB removal plots in an antagonist interaction (LRT=10, P=0.001, Table 3.1), while warming, AGB removal and N addition decreased soil moisture (LRT=47, P<0.0001; LRT=6, P=0.01; LRT=20, P<0.0001, Table 3.1).

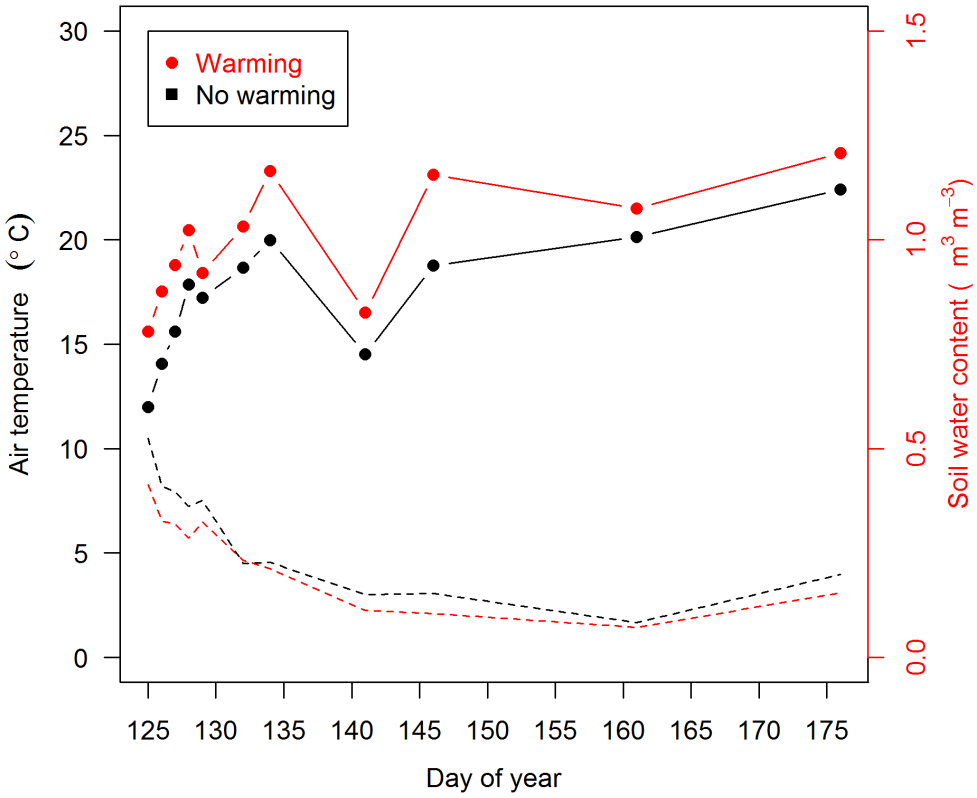

Figure 3.1 Seasonal variation in the warmed and no-warmed plots. Mean air temperature (°C), and soil water content (m^3 m^{-3}), at 100 mm depth. Solid lines represent air temperature, while dashed lines represent soil water content.

Table 3.1 Effects of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on the mean air and soil temperature, and soil moisture over May-June 2016. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P < 0.05$) are shown in bold.

		Mean air temperature °C		Mean soil temperature °C		Soil moisture %	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	168.80	**<0.0001**	1.73	0.19	47.22	**<0.0001**
AGB REMOVAL	1	0.22	0.64	10.14	**0.001**	6.10	**0.01**
NADD	1	0.16	0.69	6.87	**0.01**	19.66	**<0.0001**
WARM *x* AGB REMOVAL	1	0.77	0.38	2.75	0.10	0.75	0.38
WARM *x* NADD	1	1.17	0.28	0.10	0.76	1.44	0.23
AGB REMOVAL *x* NADD	1	0.74	0.39	0.57	0.45	10.10	**0.001**
WARM *x* NADD *x* AGB REMOVAL	1	0.01	0.92	0.20	0.65	5.54	**0.02**

3.4.2 Soil physical and chemical properties

Overall, N addition was the most important treatment affecting soil properties (Fig. 3.2). The application of N-fertiliser decreased soil bulk density and WFPS (LRT=5, P=0.02; LRT=8, P=0.005, respectively, Table 3.2). Total soil C and N was affected by an antagonistic interaction between warming and AGB removal (LRT=4, P=0.05; LRT=4, P=0.03, Table 3.2). Overall, N increased both total soil C and N (LRT=9, P=0.002; LRT=13, P=0.0004, Table 3.2).

Soil NH_4^+-N and NO_3^--N concentrations were affected by a synergistic interaction between warming and N addition (LRT=11, P=0.0006; LRT=43, P<0.0001, Table 3.2). The interactive effect of N and AGB removal decreased soil NH_4^+-N (LRT=5, P=0.02). The application of N-fertiliser increased both NH_4^+-N and NO_3^--N (LRT=20, P<0.0001; LRT=82, P<0.0001, Table 3.2) and warming increased both NH_4^+-N and NO_3^--N concentrations (LRT=6, P=0.01; LRT=11, P=0.001, Table 3.2).

The in-growth core approach was successful with minimal root biomass detected in the 35 µm and 1 µm in-growth cores relative to the 2 mm mesh core (Table 3.3). In line with reduced root biomass, soil bulk density and WFPS were significantly higher in 2 mm in-growth core followed by 35 and 1 µm mesh core (Table 3.3). Soil NO_3^--N varied in relation to in-growth cores (LRT=10, P=0.002, Table 3.2) and was higher in the 35 µm in-growth core, followed by 2 mm and 1 µm, with no significant difference between 2 mm and 1 µm in-growth core (Table 3.3). As a nonsignificant root biomass was found in the 35 and 1 µm mesh cores, the interactive effects was only analysed in the 2 mm mesh core. Root biomass was decreased by 25% (LRT=5.3, P=0.02) and 14% (LRT=5.1, P=0.02) under N addition and warming treatment, respectively (Fig. 3.3).

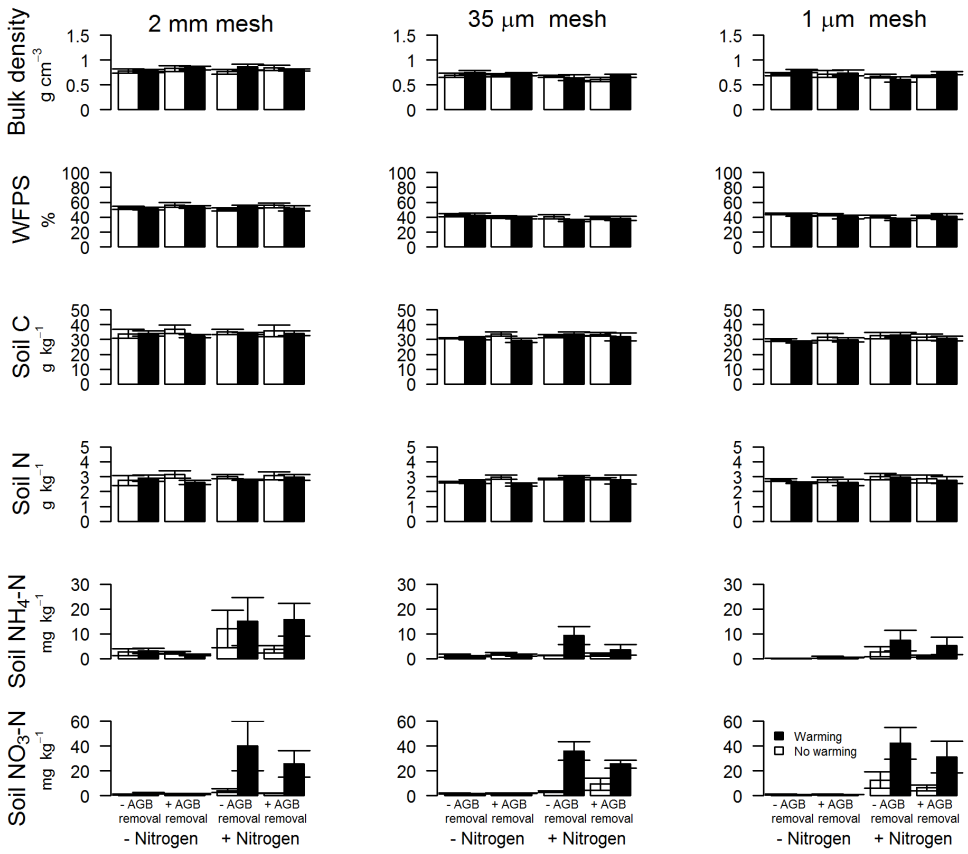

Figure 3.2 Interactive effect of warming, AGB removal, nitrogen addition and in-growth cores on bulk density, water-filled pore space (WFPS), soil C, soil N, soil NH_4^+-N and NO_3^--N. Data are means ± SE (n=5).

Table 3.2 Effects of warming (WARM), AGB REMOVAL, nitrogen addition (NADD) and in-growth core (IG) on bulk density, water-filled pore space (WFPS), soil C, soil N, soil NH_4^+-N and soil NO_3^--N. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P < 0.05$) are shown in bold.

		Bulk density		WFPS		Soil total C		Soil total N		Soil NH_4^+-N		Soil NO_3^--N	
		g cm^{-3}		%		g kg^{-1}				mg N kg^{-1} dry soil			
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	2.56	0.11	3.34	0.07	0.84	0.36	0.89	0.34	6.06	**0.01**	11.07	**0.001**
AGB REMOVAL	1	0.37	0.54	0.05	0.82	0.14	0.71	0.01	0.97	0.04	0.84	0.58	0.45
NADD	1	5.34	**0.02**	7.70	**0.005**	9.44	**0.002**	12.71	**0.0004**	20.44	**<0.0001**	82.09	**<0.0001**
IG	1	13.82	**0.0002**	106	**<0.0001**	0.00	0.96	0.28	0.59	0.36	0.54	9.64	**0.002**
WARM *x* AGB REMOVAL	1	0.02	0.87	0.18	0.67	3.73	**0.05**	4.49	**0.03**	1.94	0.16	0.97	0.32
WARM *x* IG	1	0.15	0.70	0.36	0.83	0.12	0.73	0.49	0.48	0.08	0.77	0.30	0.58
WARM *x* NADD	1	0.21	0.64	0.07	0.79	2.21	0.14	2.22	0.14	11.81	**0.0006**	43.03	**<0.0001**
NADD *x* IG	1	0.92	0.34	2.81	0.25	0.001	0.97	0.24	0.62	3.62	0.06	0.48	0.48
AGB REMOVAL *x* IG	1	0.92	0.34	3.39	0.18	0.04	0.83	0.12	0.72	0.13	0.71	1.08	0.30
AGB REMOVAL *x* NADD	1	0.30	0.58	1.64	0.20	0.99	0.32	0.99	0.32	5.28	**0.02**	0.39	0.53
WARM *x* NADD *x* AGB REMOVAL	1	1.21	0.27	0.50	0.48	0.62	0.43	2.54	0.11	0.11	0.73	2.08	0.15
WARM *x* NADD *x* IG	1	0.01	0.90	1.23	0.54	0.24	0.61	1.16	0.28	0.12	0.73	0.13	0.71
WARM *x* IG *x* AGB REMOVAL	1	0.07	0.79	1.26	0.53	0.47	0.49	0.77	0.38	1.38	0.24	0.26	0.61
IG *x* NADD *x* AGB REMOVAL	1	0.28	0.59	2.07	0.35	0.88	0.35	0.000	0.99	0.44	0.50	1.35	0.25
WARM *x* NADD *x* AGB REMOVAL *x* IG	1	0.84	0.36	2.52	0.28	0.07	0.79	0.08	0.77	3.96	0.05	3.03	0.08

Table 3.3 Bulk density, water-filled pore space, root biomass and soil NO_3^--N for in-growth cores measured at the end of the experiment.

In-growth cores	Bulk Density g cm^{-3}	Water-filled pore space %	Root biomass g m^{-2}	Soil NO_3^--N mg kg^{-1}
2 mm	0.81 ± 0.015^a	53.33 ± 0.85^a	21.04 ± 1.63^a	9.08 ± 3.06^b
35 µm	0.68 ± 0.014^b	39.89 ± 0.77^b	0.71 ± 0.15^b	9.76 ± 2.27^b
1 µm	0.71 ± 0.016^b	41.46 ± 0.77^b	0.81 ± 0.21^b	12.05 ± 3.26^a

Significant differences between treatments based on Tukey test of significance are indicated by different lowercase letters ($P < 0.05$). Data are means ± SE (n=5).

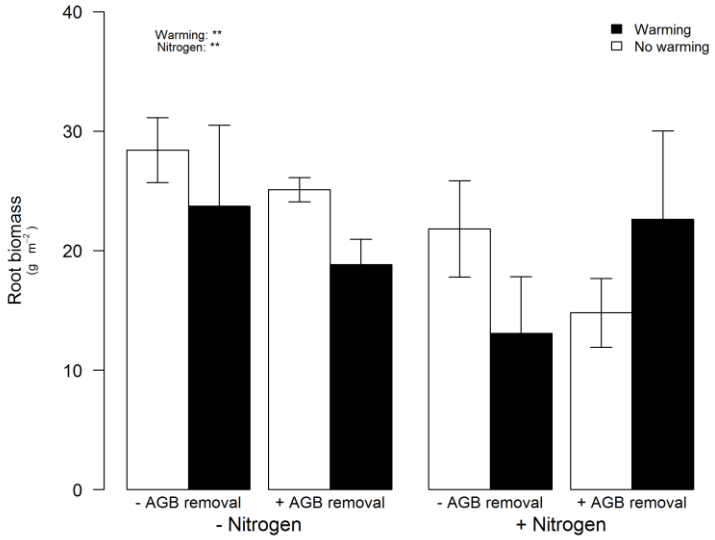

Figure 3.3 Interactive effect of warming, AGB removal and nitrogen addition on root biomass. Bars indicate mean ± SE (n=5). The significance of effects indicated by ** = P<0.05.

3.4.3 GHG emissions from the in-growth cores

Soil respiration fluxes across all treatments and in-growth cores varied over time from 37 to 174 mg CO_2-C m^{-2} h^{-1}. Soil respiration was affected by a synergistic interaction between warming and AGB removal (LRT=5, P=0.03, Table 3.4), and N addition increased ecosystem respiration (LRT=7, P=0.01, Table 3.4). Soil respiration showed significant differences between in-growth cores (LRT=16, P=0.0001, Table 3.4), with 2 mm in-growth cores had exhibiting highest CO_2 fluxes followed by 35 µm and then by the 1 µm in-growth cores (Fig. 3.4).

N_2O fluxes across all treatments and in-growth cores varied over time from 34 to -0.9 µg N_2O-N m^{-2} h^{-1}. Application of N-fertiliser induced an increase of N_2O emissions overall (LRT=49, P<0.0001, Table 3.4), and there was no significant difference between in-growth cores (LRT=0.27, P=0.60, Table 3.4, Fig. 3.4). Emissions of CH_4 across all treatments and in-growth cores varied across sampling occasions from -51 to 3 µg CH_4-C m^{-2} h^{-1}. Warming had an effect by reducing CH_4 uptake by the soil in all in-growth cores (LRT=5, P=0.03, Table 3.4). Emissions of CH_4 showed a significant difference between mesh sizes (LRT=5, P=0.03, Table 3.4), and 2 mm and 35 µm in-growth cores were not different but higher than 1 µm in-growth core (Fig. 3.4).

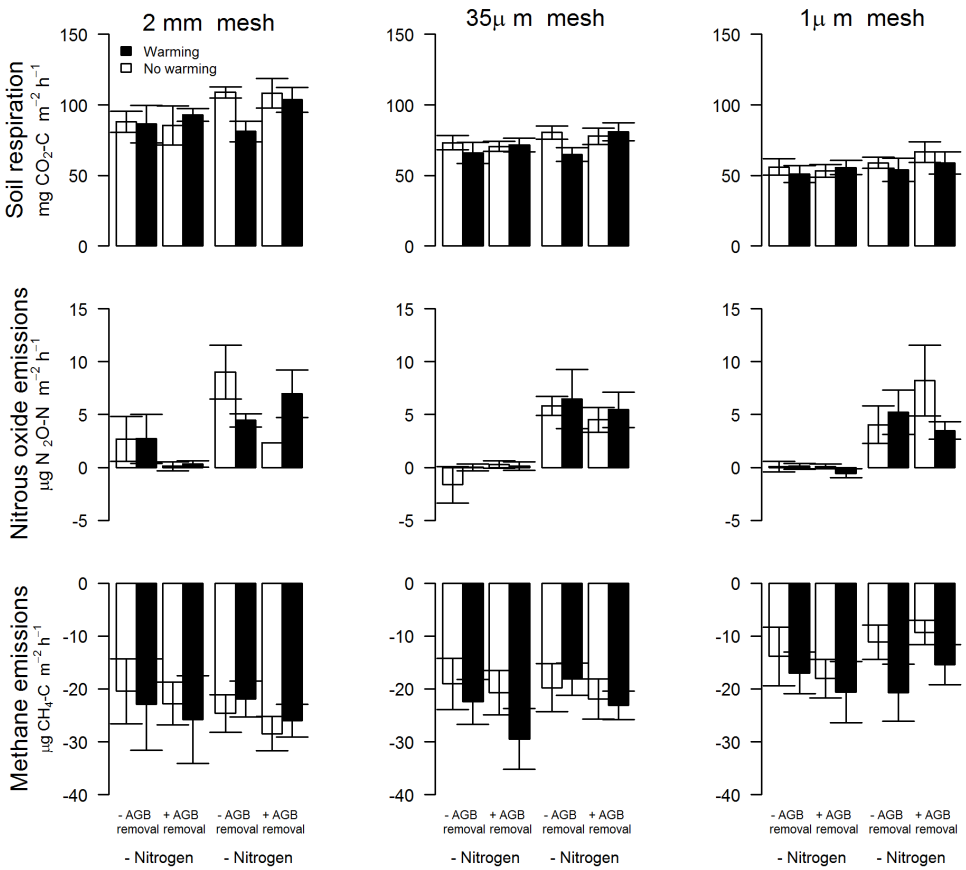

Figure 3.4 Interactive effect of warming, AGB removal, nitrogen addition and in-growth cores (2 mm, 35 µm and 1 µm mesh) on soil respiration (mg CO_2-C m^{-2} h^{-1}), nitrous oxide emissions (µg N_2O-N m^{-2} h^{-1}) and methane emissions (µg CH_4-C m^{-2} h^{-1}). Data are mean for all sampling dates ± SE (n=11).

Table 3.4 Effects of warming (WARM), AGB REMOVAL, nitrogen addition (NADD), in-growth cores (IG) and all interactions on mean CO_2, N_2O and CH_4 emission. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P < 0.05$) are shown in bold.

		CO_2 emissions mg CO_2-C m^{-2} h^{-1}		N_2O emissions µg N_2O-N m^{-2} h^{-1}		CH_4 emissions µg CH_4-C m^{-2} h^{-1}	
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	4.31	**0.04**	0.20	0.65	4.78	**0.03**
AGB REMOVAL	1	3.65	0.06	1.02	0.31	2.11	0.15
NADD	1	6.67	**0.01**	49.28	**<0.0001**	1.36	0.24
IG	1	16.41	**0.0001**	0.27	0.60	4.95	**0.03**
WARM *x* AGB REMOVAL	1	4.86	**0.03**	0.30	0.58	0.11	0.74
WARM *x* IG	1	0.00	0.97	0.55	0.46	0.18	0.67
WARM *x* NADD	1	1.34	0.25	0.24	0.62	0.23	0.63
NADD *x* IG	1	0.00	0.98	0.16	0.69	0.02	0.87
AGB REMOVAL *x* IG	1	0.00	0.96	3.61	0.06	1.03	0.31
AGB REMOVAL *x* NADD	1	1.90	0.17	0.00	0.98	1.10	0.30
WARM *x* NADD *x* AGB REMOVAL	1	0.17	0.68	0.00	0.96	0.27	0.60
WARM *x* NADD *x* IG	1	0.21	0.65	0.69	0.41	2.36	0.12
WARM *x* IG *x* AGB REMOVAL	1	1.25	0.26	0.63	0.43	1.00	0.32
IG *x* NADD *x* AGB REMOVAL	1	0.03	0.87	1.20	0.28	0.79	0.37
WARM *x* NADD *x* AGB REMOVAL *x* IG	1	1.01	0.31	0.33	0.57	0.01	0.93

3.4.4 Partitioned soil respiration

Respiration fluxes from the below-ground components varied over time with higher emissions from soil only compared to mycelial and root cores (Fig. A2.4, Appendix 2). The partition of soil respiration across all treatments showed that 61% ± 2.3 (56.4 mg m^{-2}) of the ecosystem respiration was due to the basal contribution, 22% ± 1.3 (22 mg m^{-2}) from roots, and 20% ± 2.0 (18 mg m^{-2}) from mycelia (Fig. 3.5). Basal respiration was higher, and root and mycelial respiration were not statistically different from each other (P>0.05) i.e. basal>root rhizosphere=mycelial respiration.

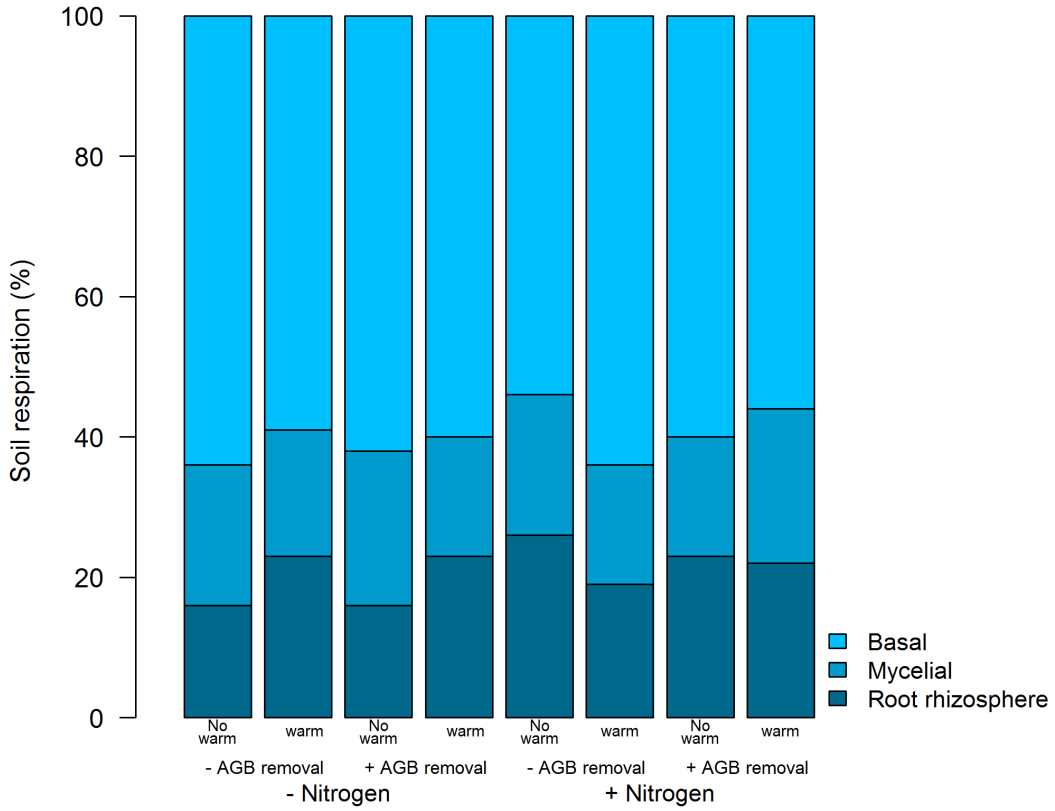

Figure 3.5 Relative contribution of root rhizosphere, mycelial and basal respiration on the total soil respiration in response to the interactive effect of warming, AGB removal and nitrogen addition. Bars represent the percentage of total below-ground respiration flux for each main treatment. No warm=no warming, warm=warming.

As there is only difference between partitioned below-ground and no treatments effects (Table A2.1, Appendix 2), ecosystem respiration was analysed in each below-ground compartment separately (Fig. 3.6, Table 3.5). Main treatments had a range of effects on partitioned soil respiration (Fig. 3.6). Root respiration was increased by warming in plots without N addition, in a synergistic interaction (LRT=4, P=0.04, Table 3.5). Mycelial respiration was marginally affected by the three-way interaction between warming, AGB removal and N addition (LRT=4, P=0.04, Table 3.5). Basal respiration was reduced by warming (LRT=5, P=0.03, Table 3.5) and increased by AGB removal (LRT=4, P=0.03, Table 3.5).

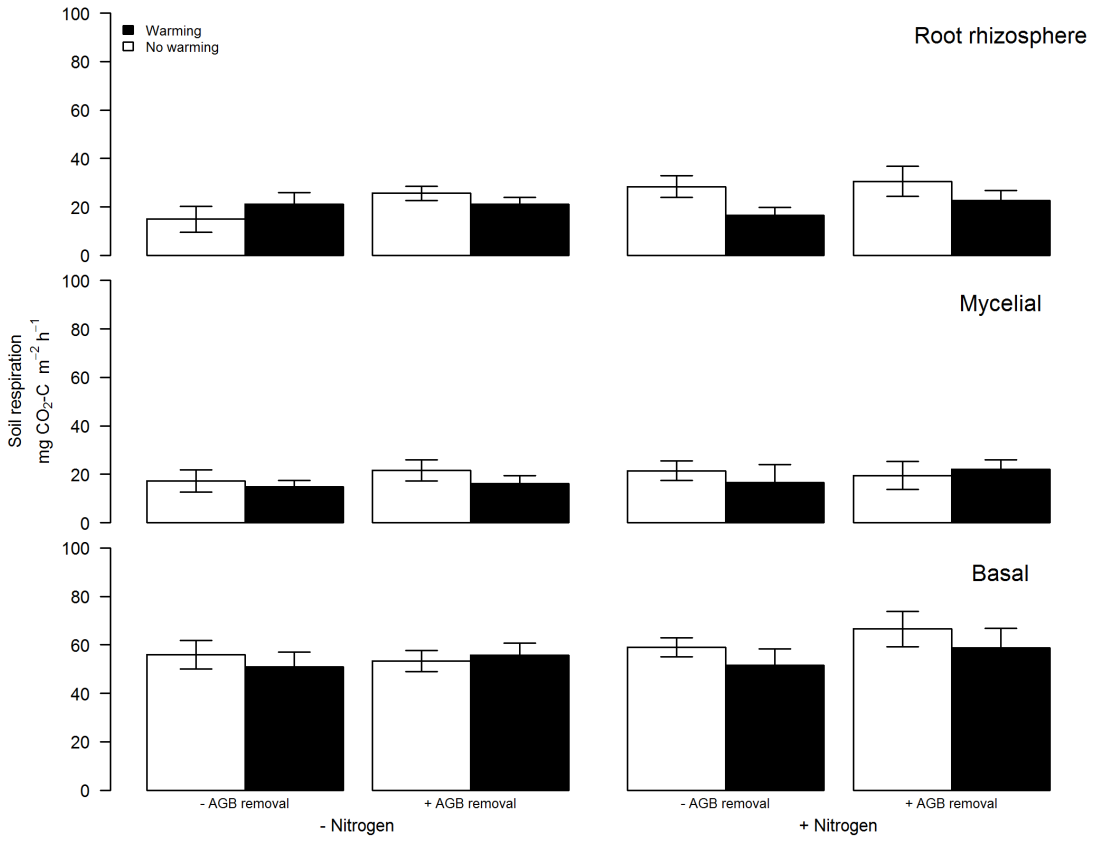

Figure 3.6 Interactive effect of warming, AGB removal and nitrogen addition on the root rhizosphere, mycelial and basal respiration. Data are means for all sampling dates ± SE (n=11).

Table 3.5 Effects of warming (WARM), AGB REMOVAL and nitrogen addition (NADD) on the root rhizosphere, mycelial and basal respiration. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effects ($P < 0.05$) are shown in bold.

		Root rhizosphere respiration		Mycelial respiration		Basal respiration	
		mg CO_2-C m^{-2} h^{-1}					
	d.f.	LRT	*P*	LRT	*P*	LRT	*P*
WARM	1	2.18	0.14	0.68	0.41	4.74	**0.03**
AGB REMOVAL	1	2.50	0.11	0.99	0.32	4.56	**0.03**
NADD	1	1.75	0.18	0.42	0.51	2.44	0.12
WARM *x* AGB REMOVAL	1	0.24	0.62	1.84	0.17	0.05	0.81
WARM *x* NADD	1	3.92	**0.04**	0.17	0.67	1.02	0.31
AGB REMOVAL *x* NADD	1	0.02	0.90	0.15	0.69	1.06	0.30
WARM *x* NADD *x* AGB REMOVAL	1	1.67	0.19	4.26	**0.04**	0.41	0.52

3.5 Discussion

3.5.1 Partitioned soil respiration from grassland

Mycorrhizae have been widely studied, however it is still not fully understood their importance on the terrestrial ecosystems (Pendall et al., 2004), despite evidence of their key role e.g. on the soil C and N cycling (Heinemeyer et al., 2007, Nottingham et al., 2010). Besides mycorrhizae are also related to mediate changes in soil structure and plant nutrient foraging (Rillig et al., 2002a, Staddon et al., 2002). This work showed that mycelial respiration contributed 20% of total grassland soil respiration during the growing season, similar to root respiration (22% of total respiration, Fig. 3.5). With these in-growth cores being left in the ground for one year prior to commencing GHG measurements (mycelia growth rate is 10 mm day^{-1}; Donnelly et al. (2004), Leake et al. (2004)), it is likely that hyphae would have sufficiently grown to reflect mycelial respiration of undisturbed soils (Nottingham et al., 2010). There are only a few studies, which partition soil respiration in grasslands, and they found similar contribution of mycorrhizae and roots (27% and 11 % of mycorrhizal and root respiration, respectively, Heinemeyer et al. (2012)). Experiments in forests, using in-growth cores method, found higher mycelial contribution relating to 25% (ectomycorrhizal: 26 mg C m^{-2} h^{-1}, Heinemeyer et al. (2007)) and 14% of total soil respiration (AMF: 17.3 mg C m^{-2} h^{-1}, Nottingham et al. (2010)). Our results are in line with these studies despite being made in different ecosystems with mycorrhiza contributing to respiration in equal measure to roots. Estimates of mycelial and root-rhizosphere respiration may be subject to several sources of error e.g. mycelia (especially AMF in grasslands) are also found inside roots (counting for an estimated 20% of root weight, Smith and Read (2008)). Moreover, for the same reason, root respiration might be overestimated. In addition, the in-growth core method does not account for differences in the presence of any mesofauna (e.g. earthworms, mites, collembolans) in the 2 mm mesh, which could promote respiration. Even so, in-growth cores techniques are recognised to be an accurate and a simple approach to make these measurements. These results show the great importance of mycorrhizae fungi on the C cycle, emitting CO_2 approximately at the same amount of root-only.

Basal respiration (microbial or heterotrophic respiration) represented the largest proportion of respiration accounting for 61 ± 2.3% of total respiration, while Graham

et al. (2014) and Zhang et al. (2014a) studying a grassland ecosystem found 71% and 50%, respectively. In a forest ecosystem, Heinemeyer et al. (2007) found a contribution of 65% of total respiration. Microbes can be responsible for the decomposition of both recent and older C in soil organic matter and by rhizosphere priming (heterotrophic respiration) (Dijkstra et al., 2013a, Ryan and Law, 2005, Trumbore, 2000). Again, our measures of basal respiration may be overestimated as we assumed that our control core (1 µm mesh) contained exclusively microbes.

Although there is no significant effect of below-ground component on the total soil C and N (Fig. 3.2), studies suggest that mycorrhizae has a great contribution on the C sequestration in the soil, due to the production of chitin, and in particular in AMF fungi, glomalin production (approx. 30-60% of C in soil) (Treseder and Allen (2000). Johnson et al. (2002a) also showed a highly dependency of AMF on photosynthetic activity; approximately 4-6% of photo-assimilates were from the mycelium respiration. Nevertheless, the short duration of the experiment may be the reason for a lack of significant differences on the total soil C and N.

3.5.2 N_2O and CH_4 emissions in the presence of root and/or mycelium

Only a few studies have examined the effect of soil below-ground components on N_2O fluxes (Bender et al., 2015, Bender et al., 2014, Veresoglou et al., 2012). During the growing season, N_2O emissions did not differ between below-ground inputs. It has been suggested that mycelium associated to roots might decrease the N availability in the soil and reduce N_2O emissions (Bender et al., 2015, Rillig, 2004). Although soil NO_3^--N decreased in the presence of root and/or mycelium (Table 3.3), it did not affect the N_2O emission. The reason may be due to a decrease in WFPS (below 40%) in the cores without roots (Table 3.3), affecting the nitrification process of N transformation in the soil (Ussiri and Lal, 2012). Likewise, Lazcano et al. (2014) stated that more important than the N uptake by mycorrhiza is water uptake, with decreases in soil moisture limiting N_2O-formation via denitrification (an anaerobic process). In addition, Holz et al. (2016) suggest that roots associated with mycorrhiza significantly decrease N_2O emissions due to an input of labile C accelerating N turnover and microbial activity.

There is no evidence to date in the literature about the effect of the presence of root and/or mycelium on the CH_4 emissions from the soil. However, it is very unlikely that

mycorrhizal fungi alter the production or either consumption of CH_4 from soils directly, although there is a possibility for indirect effects. AMF fungi can change soil structure (soil aggregation), water retention and C, N and phosphorous status of soils (Hodge et al., 2010) due to the production of glomalin which is a glycoprotein (Rillig and Mummey, 2006). In addition, with the respiration of mycorrhiza, oxygen availability can be reduced increasing anaerobic microsites in the soil, changing the balance between methanotrophic and methanogenic organisms, potentially altering the soil grassland emissions of CH_4. This can explain the reduction of CH_4 uptake in soils where root and/or mycelium were present (Table 3.4). Despite these possible effects, there is no difference on bulk density and WFPS on the 35 and 1 µm mesh core suggesting that the lack of roots in the cores were leading these differences. The presence of roots enhance soil porosity as well as soil aggregation through entanglement of particles, adhering them together (Six et al., 2004, Tisdall and Oades, 1982). Furthermore, it is very likely that other controlling factors were responsible for the reduction of CH_4 uptake in soils where root and/or mycelium were present, such as C and oxygen availability (Hodge et al., 2010), and soil pH (Li et al., 1991).

3.5.3 Effect of warming, N addition and AGB removal on soil respiration partitioning

A significant climate effect was observed with warming decreasing basal respiration and reducing the soil CH_4 uptake (Table 3.4), whilst diminishing soil NO_3^--N availability (Table 3.2) and root biomass (Fig. 3.3). Several studies suggest an increase in soil respiration and component autotrophic and heterotrophic sources under warming scenarios (Rustad et al., 2001). There are several explanations for the reduction of soil basal respiration in our study. Firstly, the warming effect might be transient (Luo et al., 2001, Melillo et al., 2002), with longer-term ecosystem acclimation explained by reduction of the root respiration rate (Burton et al., 2008) due to a reduced root biomass (Fig. 3.3, Zhou et al. (2011)). Secondly, an indirect effect of warming leading to drier soil (Fig. 3.1, Table 3.1), limiting soil respiration (Pendall et al., 2004). Thirdly, soil warming may have led to an increase in N-mineralisation and higher NO_3^- leaching or immobilisation (Table 3.2) affecting microbial respiration (Fig. 3.5c) due to limited labile C supply (Hillstrom et al., 2010). Lastly, warming could have forced the conversion of a portion of the CO_2 to CH_4 (Pendall et al., 2004), explaining a reduction

of respiration and a reduction of CH_4 uptake from the soil (Table 3.4), although it is an unlikely mechanism, unless the soil were very wet.

Although evidenced in some studies (Rustad et al., 2001) and in one of our hypothesis, increases in temperature did not affect mycorrhizal respiration. In a review by Mohan et al. (2014), warming showed a decrease in mycorrhizae activity (71% of studies), although mycorrhizae abundance was found to be increased in 63% of the evaluated studies. The lack of response on the mycorrhizae respiration could be attributed to an indirect effect of warming. Warming is supposed to increase net N mineralisation (Melillo et al., 2011, Rillig et al., 2002a), thus causing a warming-induced indirect "fertilisation effect" (Mohan et al., 2014). In this way, AMF fungi can be "inhibited" by fertilisation (Blanke et al., 2012), as N availability increases in the soil, grassland plant hosts became less dependent on mycorrhizae for N acquisition (Mohan et al., 2014). Again, contrary to our hypothesis, N addition did not affect soil respiration in either of the soil components. Lilleskov et al. (2011) suggest that AMF abundance is not consistently affected by increased N availability, although N addition is highly related to increase of plant productivity. The direct role of mycorrhizae on increases in productivity is not clear. Nevertheless, a three-way interaction between warming, N addition and AGB removal was found in our study (Table 3.5), suggesting that warming in some way interacted to grassland management, affecting mycorrhizal respiration.

As reported in many experiments, AGB removal or clipping negatively affects total soil respiration (Bremer et al., 1998, Wan and Luo, 2003, Zhou et al., 2007) however, its effect may differ in each below-ground component. As hypothesised, the short-term effect of clipping is evidenced in the basal respiration rather than root-rhizosphere respiration. The main reason is that roots have more carbohydrate reserves to continue the metabolism under limited C supply to the system (Bahn et al., 2006), thus root-rhizosphere respiration may not be affected by clipping in a temperate grassland. The effect of AGB removal on the basal respiration may be due to the availability of fresh C (derived from rhizodeposition) affecting microbial decomposition of soil organic matter (Fontaine et al., 2004, Kuzyakov, 2002, Subke et al., 2004) and/or due to an increase of soil respiration affected by clipping (Table 3.5). Thus, according to McSherry and Ritchie (2013), grazing intensities, grazing duration or climatic conditions can be reasons of variable results of different studies.

Contrary to our hypothesis, an interaction between warming and N negatively affected root respiration in our experiment (Fig. 3.5), with no effects for mycorrhizal and microbial respiration. Graham et al. (2014), studying the effect of N and warming in grassland, found an additive effect, as warming drove the interactive effect. In our study, warming could have induced the mineralisation of the N added to the system and increased root N uptake, lowering soil mineral-N (Fig. 3.2). This could led to a C limitation to the system, reducing root respiration. As discussed before, the increase of N availability in the soil leading by the interaction between warming and N could be inhibited the mycorrhizal respiration, showing no effect (Table 3.5). Although a reduction in root respiration was found in the below-ground compartment level, warming and N increased total soil respiration in the ecosystem level (Chapter 2, Fig. 2.2).

Warming interactions with AGB removal did not result in significant changes in partitioned grassland soil respiration and agreed with findings from Zhou et al. (2007) and Chapter 2. Zhou et al. (2007) investigated the effect of warming (2 °C) and yearly clipping over 5-years in tallgrass prairie ecosystem, and did not find an interaction effect between warming and cutting on the soil respiration contribution. However, our study found that this interaction reduced total soil C and N (Fig. 3.2) which might be explained by a reduction of canopy photosynthesis, slowing the translocation of C to the rhizosphere, counteracting the effect of warming. Recently, Wang et al. (2017a) found that grazing over the growing season (warmed conditions) did not affect soil respiration and its components over 5 years measurements, however, cold-grazing occurred on the non-growing season enhanced autotrophic (23.2%) and heterotrophic (4.9%) respiration. In this study, the authors suggest that the decrease of AGB due to grazing might be offset the increase of temperature over the growing season.

The interaction between AGB removal and N addition did not affect either any of the soil component respiration. To date, there is no study, which evaluates this interaction in the field, thus it is very difficult to predict responses to these changes. Despite this, it was observed an increase of soil moisture (Table 3.1) under the interaction between AGB removal and N addition. Studies suggest that extremes in soil moisture, high or low, may result in a reduction of root and/or microbial respiration, which consequently will inhibit soil respiration (Wang et al., 2003, Xu et al., 2004). This may be the reason

by which the interaction between AGB removal and N addition did not affect soil respiration in the compartment level. Besides, Kuzyakov et al. (2002) found that after cutting CO_2 efflux was lowered in the fertilised compared to unfertilised plants in a 55 days incubation experiment. The authors then suggest that N fertilisation might lower the C losses, especially during the regrown plants after cutting (due to reduction of C assimilation), limiting soil respiration.

3.6 Conclusions

This study demonstrates that microbial respiration accounted for 61% of grassland soil respiration, whilst mycorrhizal respiration represents also a great contribution on the total soil respiration (20%) similar to root respiration (22%). The contribution of soil respiration did not differ under interactions between climate warming and grassland management. Warming may have an effect in promoting the acclimation of soil below-ground respiration as well as of the ecosystem respiration (Chapter 2), due to the decrease of basal respiration and decreased soil CH_4 uptake, while also decreasing soil NO_3^- availability. There were no differences in N_2O fluxes between below-ground compartments. However, CH_4 uptake was decreased in the presence of both roots and mycelia compared to control plots (1 µm mesh cores), mainly due to changes in soil structure and microclimatic conditions. Warming interacted with N addition decreasing root-rhizosphere respiration and mineral-N in the soil. Warming and AGB removal did not affect soil respiration and its components while reduced nutrients input to the soil. Overall findings show that, despite important individual effects, interactive effects of climate warming and management practices are often complex and difficult to predict. Additionally, soil N_2O and CH_4 fluxes were found to be less responsive to changes in ecosystem drivers, as well as in the soil components, than respiration. Future experiments will need to include longer temporal and larger spatial scales (world temperate grasslands), different intensities of cutting, N addition rates and degrees of warming, including grazing events which might differ due to trampling, dung and urine deposition in soil. Grassland management, e.g. which affect nutrient availability in soils, and soil structure, will be likely to impact on below-ground compartments, with direct responses on C and N cycling, and GHG emissions from soils. Improved knowledge of the effects of interactive drivers of change on grassland C and N and

GHG fluxes and their sources is needed to improve modelling and forecasting of feedbacks to and from the climate system.

4 Effects of legume-fertiliser N interactions on C and N cycling in grass-legume mesocosms

Arlete S. Barneze [1,2], Jeanette Whitaker [2], Niall P. McNamara [2] and Nick J. Ostle [1]

[1] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

[2] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK.

4.1 Abstract

Grasslands are important production systems with functions and services that are affected by interactions between different land management practices. In temperate environments, grass-legume mixtures are commonly employed to increase productivity via symbiotic N_2 fixation by legumes, reducing the need for nitrogen (N) fertiliser. The aim of this study was to investigate the interactions between the proportion of legumes in grass-legume mixtures and N-fertiliser addition on carbon (C) and N cycling, including greenhouse gas (GHG) emissions. The hypotheses were that i) an increase in the relative proportion of legumes will increase plant productivity and decrease GHG emissions, and ii) the magnitude of these effects will be reduced by N addition. To test these hypotheses a mesocosm experiment was conducted in a controlled temperature room with one grass and one legume species grown in mixtures in different proportions, with or without N-fertiliser addition. The effects on C and N cycling processes in grass-legume mixtures were assessed by measurement of above- and below-ground plant productivity and biomass, total soil C and N, soil mineral-N, N mineralisation and nitrification, shoot N uptake and GHG emissions (CO_2, N_2O and CH_4). We found no effect of legume proportion in fertilised and unfertilised soil on N_2O emissions, contrary to the hypothesis. However, ecosystem respiration was decreased by an increase in legume proportion only in the fertilised soils, probably relating to a reduction of below-ground biomass. CH_4 emissions decreased non-linearly with legume proportion. Plant productivity and above- and below-ground biomass had a non-linear relationship with relative legume proportion. Total soil N and mineral-N were not affected by increases in relative legume proportion. This study shows that the proportion of legumes in grass-legume mixtures affected plant productivity above and below-ground, and the C cycle (CO_2 and CH_4 emissions), however soil properties and N cycle were unaffected in this

short-term experiment. Improving our understanding of the individual and interactive effects of legumes and N addition on GHG emissions and C and N stocks is important to inform the development of grassland management strategies to mitigate climate change.

Keywords: species mixtures, legumes, N-fertiliser, C and N cycling, plant productivity, GHG emissions.

4.2 Introduction

Grasslands provide important ecosystem services worldwide. These include, but are not limited to, sequestering carbon (C) in plant biomass and soil organic matter and producing the majority of forage for ruminants (De Deyn et al., 2008). Grassland species composition has been shown to be an important driver of these ecosystems functions (Bardgett, 2011, De Deyn et al., 2009, Loreau and Hector, 2001, Tilman et al., 2001) due to plant-plant and plant-soil interactions. The plant-diversity-productivity relationship can affect plant-soil feedbacks, e.g. direct effects of soil resource availability through combination and availability of mineral nutrients in the soil. While the effects of grassland species composition has been widely studied in relation to a range of ecosystem services, its influence on greenhouse gas (GHG) emissions remains largely unexplored, especially for nitrous oxide (N_2O) and methane (CH_4) (Abalos et al., 2014, Abalos et al., 2018, Niklaus et al., 2006, Sun et al., 2013).

Grass-legume mixtures are commonly used for grazing because they offer the potential to increase productivity without the use of costly and polluting nitrogen (N) fertilisers. Legumes provide high quality forage due to their high fibre and protein content (Mortenson et al., 2004). More importantly, they promote symbiotic N_2 fixation, being able to utilise atmospheric N_2 for their requirements and thereby produce more protein with less N inputs (Suter et al., 2015). N_2 fixation by legumes can be substantial ranging from 100 to 380 kg ha^{-1} y^{-1} in northern temperate regions (Ledgard et al., 2001). In grass-legume mixtures, legumes acquire 80% of their N requirement via symbiosis (Boller and Nösberger, 1987, Carlsson et al., 2009, Oberson et al., 2013) transferring and making N available for grasses, thus reducing plant competition for nutrients and diminishing the need for inorganic N-fertiliser additions. The use of mixtures has also been shown to increase plant community productivity due to increases in the resource

uptake efficiency by: i) N transfer from legumes to grasses (Høgh-Jensen and Schjoerring, 1997, Pirhofer-Walzl et al., 2012), and/or ii) due to species differences in root structure increasing the exploitation of soil resources (Mueller et al., 2013, van Ruijven and Berendse, 2005). Besides decreasing the use of N-fertiliser and energy use in the system, legume-based cropping could also contribute to the reduction of C and N losses, potentially improving the global nutrient balance (Drinkwater et al., 1998, Li et al., 2016) and increasing C sequestration (De Deyn et al., 2011). Increases in legume proportions in grass-legume mixtures may, therefore, affect plant productivity (Nyfeler et al., 2011, Suter et al., 2015) and influence plant-soil GHG emissions, something scarcely addressed in experimental studies.

The effect of grass-legume mixtures on GHG emissions from soil is likely to differ in unfertilised and fertilised soils, due to changes in mineral-N availability in the soil (Oertel et al., 2016), but also C availability for microbes, soil pH, and microbial community composition (Bardgett et al., 1999, Thomson et al., 2012). In unfertilised soils, legumes may enhance plant productivity, reducing the mineral-N available in the soil with effects on GHG emissions, especially for N_2O emissions. In addition, biological N fixation in an unfertilised soil is considered a small source of N_2O emissions (Carter and Ambus, 2006). Contrary, increases in legumes would increase the symbiotic N_2 fixation, liberating N-mineral (Niklaus et al., 2006), augmenting N_2O emissions from soil. Thus, the effect of increases in legume proportions on N gases release to the atmosphere will potentially reflect the balance between N uptake by plants and the mineral-N availability in the soil.

In fertilised soils, the effect of increasing legume proportion on N_2O emissions can be contradictory. Legumes species may be suppressed by N applications, which promote the dominance of grasses (De Deyn et al., 2011, Ledgard, 2001, Smith et al., 2008b), with the effect on N_2O emissions driven by N addition rather than legume proportion itself. On the other hand, increase in legume proportion may reduce plant N uptake (due to increase N_2 fixation), leading to higher mineral-N in the soil (Niklaus et al., 2006). In addition, increased N_2-fixation by legumes may increase residues rich in N (and hence a low C:N ratio) which may be decomposed and large quantities of mineral-N may be released to the soil, increasing soil mineral-N availability and affecting nitrification and denitrification (Huang et al., 2004). Legumes could thus result in

substantial N_2O production due to residue decomposition, but emissions from the N_2-fixation itself are less certain (Rochette and Janzen, 2005).

Mixtures of grasses and legumes, besides influencing plant productivity, may alter the quality and quantity of plant inputs to the soil, affecting not only N but also C sequestration in grasslands (De Deyn et al., 2009, Fornara and Tilman, 2008). These changes may influence C cycling and ecosystem respiration, and also CH_4 uptake in grassland soils. Increases in legume proportion in the community composition is supposed to reduce microbial and root respiration (De Deyn et al., 2011), with a reduction of ecosystem respiration. Besides, changes in the proportion of legumes may also affect organic matter inputs to the soil (Mortenson et al., 2004), directly affecting CO_2 release from the system (Drinkwater et al., 1998). Given that C cycling and N are closely related, CH_4 fluxes in the fertilised soil can also be affected by grass-legume mixtures, mainly due to increases of ammonium (NH_4^+) concentration in the soil. NH_4^+ competes with CH_4 at enzymatic levels or as a competitive effect of nitrifiers and methanotrophs (Topp and Pattey, 1997). Thus, the effect of grass-legume mixtures will depend on intra- and inter-specific plant competition and facilitation, which in turn may affect biological N fixation, community productivity (Kirwan et al., 2007, Nyfeler et al., 2011) and plant-soil GHG emissions.

Improved understanding of the effects of interactions between grassland management practices on GHG emissions is required to inform sustainable production. The aim of this study was to investigate how the interaction between grassland legumes and N-fertiliser addition affects plant-soil properties, and C and N cycling, including GHG emissions, in a factorial mesocosm experiment. The following key hypotheses were tested:

In unfertilised pots, a higher proportion of legumes in grass-legume mixtures will i) increase plant productivity and ecosystem respiration, and ii) promote plant N uptake and reduce N_2O emissions as a result of lower soil N availability. In fertilised pots, N addition will iii) reduce legume biomass production whilst favouring grass productivity and increasing ecosystem respiration, and iv) contribute to higher N_2O emissions with greater soil N availability irrespective of legume biomass.

To test these hypotheses a controlled temperature experiment was conducted using one grass and one legume species grown in mixtures with different proportions, with or

without N-fertiliser application. The effects of grass-legume mixture on plant-soil C and N cycling processes were then assessed through the measurement of above- and below-ground plant productivity, soil nutrient availability and GHG emissions.

4.3 Material and methods

4.3.1 Experimental design

A controlled temperature mesocosm experiment was conducted at Lancaster Environment Centre, Lancaster University in October 2016 to quantify the effects of changing legume proportion, in a grass-legume mixture, on plant-soil C and N cycling and GHG emissions (Fig. A3.1, Appendix 3). A fast-growing legume species, *Trifolium pratense* L. (Tr), and a grass species, *Agrostis capillaris* L., were used in this experiment (both commonly occurring in managed temperate grasslands across Europe). These plant species are known to differ in their functional traits including average root diameter, specific root length, root dry matter content, leaf N content, and leaf dry matter content (Table 4.1). A factorial experiment examining the effects of legume proportion and N addition was established. Five legume-grass mixtures with 0, 25%, 50%, 75% and 100% legume abundance were superimposed with N addition giving a total of 10 treatments. N was applied at an equivalent rate of 100 kg N ha^{-1} as NH_4NO_3 (consistent with general grassland management for hay meadows in the UK). Realised legume proportion was determined using legume above-ground biomass of each mixture (Fig. 4.1). The experiment was arranged in a full-randomised design with five replicates per treatment (50 pots in total).

Table 4.1 Plant and root traits of the grass and legume species based on own measured values collected at final harvest, for non-nitrogen addition (NoNADD) and nitrogen addition (NADD). Data are mean ± SE (n=5).

Plant species	Specific root length m g^{-1}		Root dry matter content mg g^{-1}		Leaf nitrogen content mg g^{-1} DM		Leaf dry matter content mg g^{-1}	
	NoNADD	NADD	NoNADD	NADD	NoNADD	NADD	NoNADD	NADD
Agrostis capillaris	30 ± 5	31 ± 0.01	222 ± 9	223 ± 17	26 ± 2	73 ± 6	381 ± 12	361 ± 6
Trifolium pratense	22 ± 3	18 ± 0.7	204 ± 9	222 ± 14	428 ± 3	415 ± 4	342 ± 6	412 ± 17

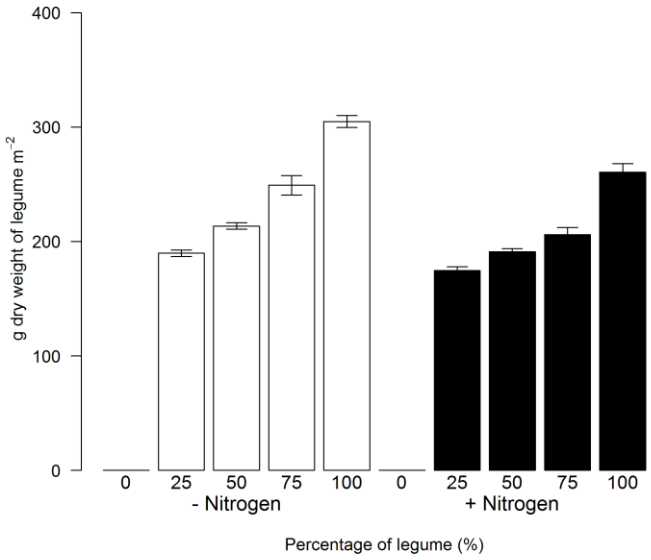

Figure 4.1 Legume above-ground biomass (g dry weight m^{-2}) within grass-legume mixtures at the end of the controlled temperature mesocosm experiment. Data are mean ± SE (n=5).

Plastic (PVC) pots (13 cm diameter and 9 cm depth) were filled with 800 g soil at 70% water-filled pore space (WFPS). Topsoil (0-10 cm depth) was collected from a permanent temperate grassland at Hazelrigg, Lancaster University, UK (54º 1'50''N, 2.7º 46'30''W, 94.1 m a.s.l.). The soil contained 25.6 g total C kg^{-1}, 2.3 g total N kg^{-1}, and had a pH (H_2O) of 5.9. Roots and plant fragments were removed, soil was sieved through 8 mm screen, thoroughly mixed and air-dried at 20 ºC to 70% WFPS. Soil moisture was gravimetrically maintained every two days in all mesocosm pots.

Seeds of *T. pratense* and *A. capillaris* were sourced from Emorsgate Seeds (Kings Lynn, UK). Prior to the start of the experiment, seeds were surface-sterilised by dipping them in diluted bleach (1% v: v) for 1 min and thoroughly rinsing them with sterile distilled water. Seeds were initially sown in sterilised potting soil (John Innes No. 2, Westland Garden Health, UK), germinated in a greenhouse and grown for four weeks, receiving water daily. The seedlings were then transplanted into pots and, after one week, dead plants were replaced. Each mesocosm pot contained a total of 12 plants. Pots were maintained in a controlled environment room (temperature, 19 °C day, 16 °C night; light between 08:00 h and 20:00 h daily).

4.3.2 Greenhouse gas fluxes measurements

Measurements of CO_2, N_2O and CH_4 were made on mesocosm pots using opaque plastic chambers (13 cm diameter and 12 cm depth) with septa to allow headspace gas sampling (Fig. A3.2, Appendix 3). For each gas flux measurement, the chamber was attached with clip seal to the mesocosm and a 10 mL headspace sample was taken immediately (t0) with a gas syringe. 5 mL of the gas was then injected into a pre-evacuated 3.5 mL exetainer vial (Labco, Lampeter, UK). Further headspace samples were taken 15 (t15) and 30 (t30) minutes after chamber closure. Chambers were then removed. Gas samples were analysed using a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) fitted with a methaniser with a Flame Ionisation Detector (FID) and Electron Capture Detector (ECD) operating at 130 °C. The GC was fitted with a stainless steel Porapak Q 50-80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. Three calibration gas standards (500 ppm, 1000 ppm, 4000 ppm CO_2) (Air Products, Waltham on Thames, UK) were run every 14 samples to enable calibration and drift correction (Case et al., 2012). Gas fluxes were calculated using linear regressions through sampling time points and were corrected for temperature and barometric pressure following the ideal gas law (Chadwick et al., 2014, Holland et al., 1999). Gases were sampled immediately after N application and daily during the first 14 days of the experiment, then three times a week up to day 40. Cumulative GHG fluxes were calculated by linear interpolation of the average GHG emissions between the measurements to integrate the fluxes over the total experimental period.

4.3.3 Plant and soil analyses

At the end of the experiment (40 days), grass and legume above-ground biomass were harvested and separated, with dry matter content determined by drying in an oven at 105 °C for 24 h. Sub-samples were dried at 60 °C, then ground and analysed for total C and N content using an elemental analyser (TruSpec® CN, St. Joseph, MI) with a furnace temperature of 950 °C. Ethylenediaminetetraacetic acid (EDTA) was used as a reference. To determine shoot N uptake per plot, values of N content (%) were multiplied by total dry matter content.

Soil was taken from the pots at the end of the experimental period. Gravimetric moisture content was determined after drying at 105 °C for 24 h. Mineral-N (NH_4^+ and NO_3^-) was extracted with 1 M KCl in a 1:5 (soil weight: extractant volume) ratio and analysed with a spectrophotometer (Auto Analyser 3 Digital colorimeter BRAN + LUBBE). Net mineralisation (net N production: NH_4^+-N + NO_3^--N) and net nitrification (net NO_3^--N production) rate were determined by incubating the soil at 25 °C for 14 days, analysing the final mineral-N content as described above, then calculating the daily mineral-N production rate as the difference between final and initial inorganic N content, divided by the incubation period. Soil C and N were determined on dried (60 °C), finely ground soil samples, using an elemental analyser (TruSpec® CN, St. Joseph, MI) with furnace temperature at 950 °C. Soil pH was measured on 10 g fresh soil with 25 mL deionised water, left to stand for 30 minutes and then tested with 210 pH Meter (Hanna Instruments, RI, USA).

Plant roots were separated from the soil from each mesocosm pot to determine below-ground biomass/productivity after washing and drying at 105 °C for 24 h. A subset of roots was stored in the fridge with 10% ethanol solution to measure specific root length (SRL) and root dry matter content (RDMC). Root length and diameter were analysed using WinRhizo® root analysis software (Regent Instruments Inc., Sainte-Foy-Sillery-Cap-Rouge, QC, Canada) coupled to an Epson flatbed scanner.

4.3.4 Statistical analyses

Generalised least squares models (GLS) were used to test the significance of treatment effects on plant productivity, plant-soil properties and GHG emissions. As most variables did not fit a linear regression, quadratic and cubic regressions were included in the model with the following formulae, where LEG refers to legume proportion and NADD to N addition:

$$y \sim LEG*NADD + I(LEG\text{^}2)*NADD + I(LEG\text{^}3)*NADD) \qquad \textit{Equation 1}$$

Fixed effects were LEG, LEG^2, LEG^3, NADD and their interactions. For all GLS models, data were checked for normality and equal variances using residual plots method, and log-transformed where necessary before analysis (i.e. for cumulative CO_2, below-ground biomass, shoot N yield, shoot C:N, shoot:root ratio, soil mineral-N, N

mineralisation and nitrification). Weight functions were used to account for unequal variances following Zuur et al. (2011). The same model parameters were found to be significant when legume proportion (%) was replaced with legume biomass (Table A3.1, Appendix 3). The proportion of legumes and biomass legumes were very highly correlated (r^2=0.80, Variance inflation factors (VIF) > 5). Additionally, the model with legume proportion showed the lowest Akaike information criterion (AIC). All statistical analysis and graphs were made using R programming language 3.4.3 (R Development Core Team, 2017) with the additional packages *nlme* (Pinheiro et al., 2017) and *plyr* (Wickham, 2011).

4.4 Results

4.4.1 Greenhouse gas fluxes

GHG fluxes over time are shown in Fig. A3.3-A3.5, Appendix 3. Cumulative CO_2 fluxes (ecosystem respiration) decreased with increases in legume proportion (P<0.0001, LEG*NADD, Table 4.2, Fig. 4.2) when N was added (Fig. A3.6, Appendix 3). Increased legume proportion did not increase cumulative N_2O emissions, regardless of N addition (P>0.05, Table 4.2, Fig. 4.2). N-fertilised treatments augmented N_2O emissions by approximately four times compared to unfertilised control treatments (P<0.0001, Table 4.2, Fig. 4.2). Cumulative CH_4 emissions were affected by legume proportion with a significant cubic regression (P=0.01, Table 4.2), and by N addition which increased CH_4 emissions by up to 50 times compared to unfertilised soils (P<0.0001, Fig. 4.2).

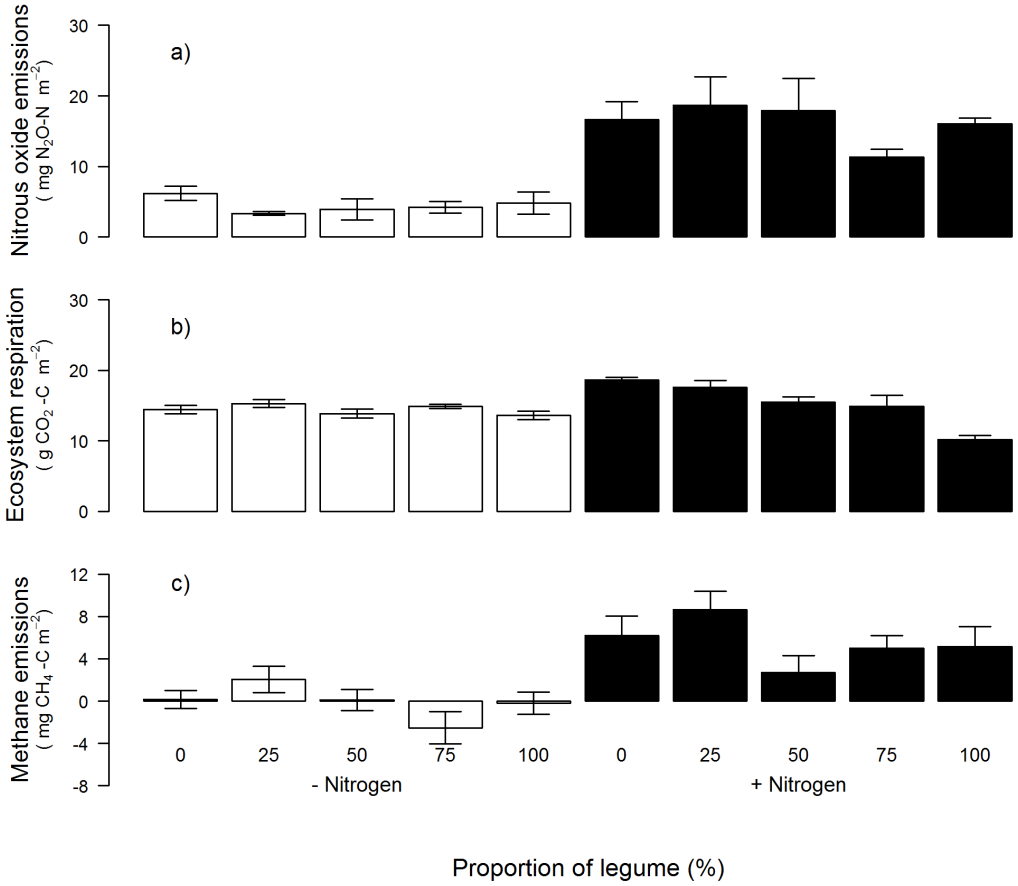

Figure 4.2 Cumulative N_2O (mg N_2O-N m^{-2}) (a), CO_2 (g CO_2-C m^{-2}) (b) and CH_4 (mg CH_4-C m^{-2}) (c) emissions in response to legume proportion and nitrogen addition in the controlled temperature mesocosm experiment. Data are mean of cumulative emissions for all sampling dates ± SE (n=18).

Table 4.2 Effects of legume proportion (LEG) and nitrogen addition (NADD) on cumulative emissions of N_2O, CO_2, CH_4. Significant effects ($P < 0.05$) are shown in bold.

		N_2O emissions mg N_2O-N m^{-2}		CO_2 emissions g CO_2-C m^{-2}		CH_4 emissions mg CH_4-C m^{-2}	
	d.f.	F value	*P*	F value	*P*	F value	*P*
LEG linear (LEG)	1	0.93	0.34	21.3	**<0.0001**	3.19	0.08
NADD	1	65.22	**<0.0001**	1.1	0.30	35.54	**<0.0001**
LEG quadratic (LEG^2)	1	1.81	0.18	3.4	0.07	0.11	0.74
LEG cubic (LEG^3)	1	0.03	0.86	0.6	0.45	7.12	**0.01**
LEG x NADD	1	0.43	0.52	26.2	**<0.0001**	0.006	0.94
LEG^2 x NADD	1	0.33	0.57	1.9	0.17	0.18	0.67
LEG^3 x NADD	1	2.83	0.10	0.9	0.35	0.15	0.69

4.4.2 Plant productivity

There was a non-linear relationship between the proportion of legumes and above-ground biomass in the unfertilised and fertilised soil (P=0.02, LEG^2*NADD, Table 4.3, Fig. 4.3a). However, fertilised soils showed higher above-ground biomass compared to unfertilised soils in all treatments except for the legume monoculture (Fig. A3.6, Appendix 3). Below-ground biomass decreased non-linearly with increases in legume proportion in both unfertilised and fertilised soil (P=0.03, LEG^3*NADD, Table 4.3, Fig. 4.3b). Nevertheless, in unfertilised soils there is a small increase when legume proportion was 25%, showing a sharp decrease with increase in legume proportions (Fig. A3.6, Appendix 3). In fertilised soils, below-ground biomass showed a constant biomass under mixtures, with decrease under legume monoculture (Fig. A3.6, Appendix 3).

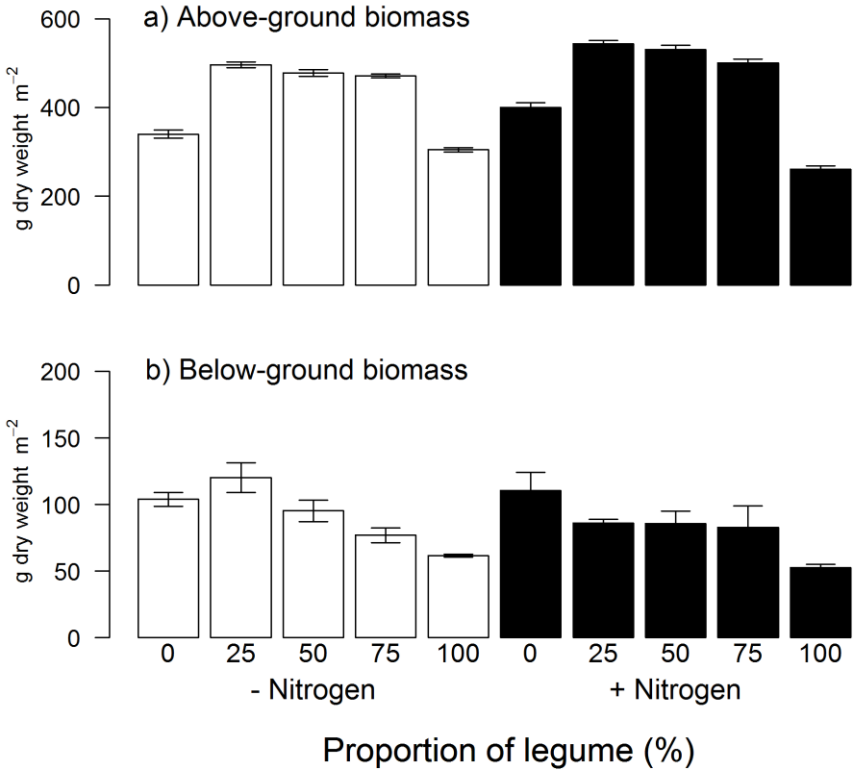

Figure 4.3 Total above- (a) and below-ground (b) biomass (g dry weight m^{-2}) in response to legume proportion and nitrogen addition in the controlled temperature mesocosm experiment. Data are mean ± SE (n=5).

Table 4.3 The effect of legume proportion (LEG), nitrogen addition (NADD) and interactions on above- and below-ground biomass. Above-ground biomass corresponds to the legume+grass biomass. Significant effects ($P < 0.05$) are shown in bold.

		Above-ground biomass g dry weight m^{-2}		Below-ground biomass g dry weight m^{-2}	
	d.f.	F value	*P*	F value	*P*
LEG linear (LEG)	1	61.70	**<0.0001**	58.96	**<0.0001**
NADD	1	14.93	**0.0004**	3.77	0.06
LEG quadratic (LEG^2)	1	511.44	**<0.0001**	5.19	**0.03**
LEG cubic (LEG^3)	1	0.57	0.45	0.15	0.70
LEG *x* NADD	1	18.29	**0.0001**	0.03	0.85
LEG^2 *x* NADD	1	5.77	**0.02**	0.37	0.54
LEG^3 *x* NADD	1	1.60	0.21	4.97	**0.03**

Shoot N uptake (g N m^{-2}) which consists of the total N uptake by grass and legumes, had a significant non-linear relationship with increasing proportion of legumes ($P<0.0001$, LEG*NADD, Table 4.4), with greater effects on fertilised soil compared to unfertilised (Fig. 4.4). In fertilised soil, shoot N uptake decreases with legumes proportion greater than 75%, while in unfertilised soil, legume proportions had a smaller effect on total shoot N uptake (Fig. A3.6, Appendix 3). Total shoot uptake reflects the changes on the above-ground biomass (Fig. 4.3a).

The N content (%) of the grass and legume decreased linearly with increases in legume proportion in the fertilised soils, but not in unfertilised treatments ($P=0.03$ and $P=0.001$, LEG*NADD, in grass and legume species respectively, Table 4.4, Fig. 4.4). The N (%) in grass and legumes was greater in fertilised soil, compared to unfertilised (Fig. A3.6, Appendix 3). Total N content in legume monoculture was 26% higher than grass monoculture in the absence of N addition, but 34% lower than grass monoculture with N addition (Fig. 4.4).

Shoot CN ratio decreased with increases in legume proportion ($P<0.0001$, LEG* NADD, Table 4.4, Fig. 4.4), only in the unfertilised soils (Fig. A3.1, Appendix 3), while shoot/root ratio were increased by increases in legume proportion ($P=0.01$, LEG* NADD, Table 4.4, Fig. 4.4), and even higher in fertilised soils (Fig. A3.6, Appendix 3).

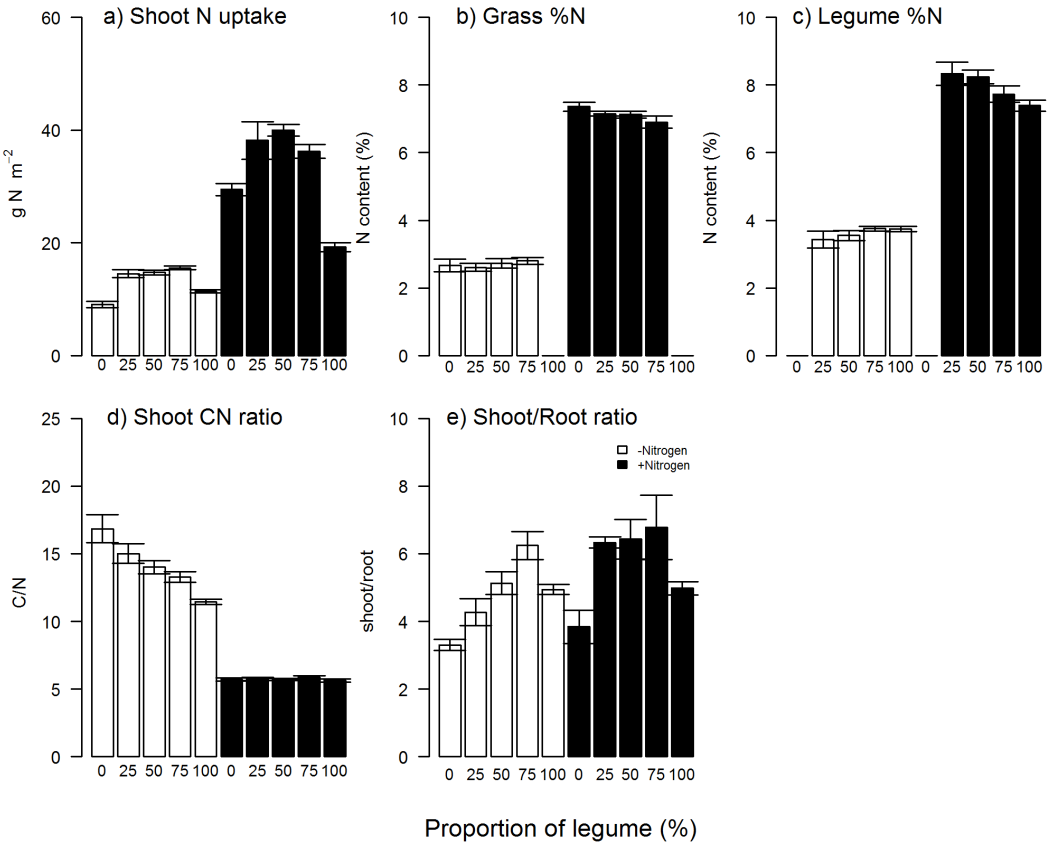

Figure 4.4 Interactive effect of legume proportion and nitrogen addition on a) total shoot N uptake, b) N content (%) in grass, c) N content (%) in legume, d) shoot CN ratio, and e) shoot/root biomass ratio in the controlled temperature mesocosm experiment. Data are mean ± SE (n=5).

Table 4.4 Effects of legume proportion (LEG), nitrogen addition (NADD) and interaction on shoot N uptake, N content in grass and legume species, shoot CN ratio and shoot/root ratio. Significant effects ($P < 0.05$) are shown in bold.

		Shoot N uptake ($g\ m^{-2}$)		N content in grass (%)		N content in legume (%)		Shoot CN ratio		Shoot/Root biomass ratio	
	d.f.	F value	*P*	F value	*P*	F value	*P*	F value	*P*	F value	*P*
LEG linear (LEG)	1	21.04	**<0.0001**	1.10	0.30	0.21	0.65	8.35	**0.006**	14.0	**0.0005**
NADD	1	1401.12	**<0.0001**	2238.94	**<0.0001**	1124.8	**<0.0001**	1948.7	**<0.0001**	4.12	**0.05**
LEG quadratic (LEG^2)	1	307.83	**<0.0001**	0.10	0.76	0.70	0.41	0.84	0.36	32.75	**<0.0001**
LEG cubic (LEG^3)	1	12.53	**0.001**	0.50	0.48	0.02	0.88	1.72	0.20	0.47	0.50
LEG x NADD	1	70.77	**<0.0001**	5.30	**0.03**	12.94	**0.001**	36.95	**<0.0001**	6.41	**0.01**
LEG^2 x NADD	1	8.17	0.007	0.17	0.68	0.01	0.91	0.00	0.99	1.94	0.17
LEG^3 *x* NADD	1	1.87	0.18	0.04	0.85	0.55	0.46	0.33	0.57	1.22	0.14

4.4.3 Soil parameters

Soil pH was not affected by increases in legume proportion (P>0.05, Table 4.5) but it was significantly affected by N addition (P<0.05), decreasing soil pH from an average value of 6.1 to 5.4 (Table 4.5). Total soil C was non-linearly affected by legume proportion (P=0.04, LEG^3*NADD, Table 4.5), mixtures showed higher total soil C in fertilised soils compared to unfertilised (Fig. A3.6, Appendix 3). Total soil N, NO_3^--N and net nitrification rates were not significantly affected by legume proportion (P>0.05, Table 4.5), but were greater under N addition (P<0.05, Table 4.5). Soil NH_4^+-N had a non-linear relationship with increasing legume proportion (P=0.001, LEG^2*NADD, Table 4.5) but only in fertilised treatments (Fig. A3.6, Appendix 3). Net mineralisation rate increased non-linearly with increases in legume proportions (P=0.006, LEG^3*NADD, Table 4.5), on the fertilised soils only (Fig. A.3.6, Appendix 3).

Table 4.5 Effects of legume proportion (LEG), nitrogen addition (NADD) and interaction on soil parameters. Data are mean ± SE (n=5). Significant effects (P < 0.05) are shown in bold.

	Soil pH	Total soil C	Total soil N	Soil NH_4^+-N	Soil NO_3^--N	Net mineralisation rate	Net nitrification rate
		mg kg^{-1}		mg kg^{-1}		mg kg^{-1} d^{-1}	
Without nitrogen							
0% Legume	6.04 ± 0.17	25.87 ± 2.08	1.89 ± 0.20	1.00 ± 0.09	0.43 ± 0.22	0.48 ± 0.08	0.39 ± 0.07
25% Legume	6.2 ± 0.10	27.00 ± 1.97	2.20 ± 0.25	0.84 ± 0.04	0.45 ±0.67	0.54 ± 0.14	0.44 ± 0.13
50% Legume	6.2 ± 0.04	29.39 ± 0.32	2.10 ± 0.11	0.92 ± 0.10	0.30 ±0.12	0.60 ± 0.09	0.52 ± 0.08
75% Legume	6.0 ± 0.14	25.59 ± 1.69	1.96 ± 0.19	0.87 ± 0.05	0.46 ± 0.18	0.50 ± 0.05	0.42 ± 0.04
100% Legume	6.2 ± 0.07	30.21 ± 1.23	2.25 ± 0.15	1.20 ± 0.11	0.83 ±0.29	0.56 ± 0.07	0.51 ± 0.06
With nitrogen							
0% Legume	5.4 ± 0.11	28.66 ± 1.00	2.41 ± 0.06	296.5 ± 9.40	109.20 ± 8.80	2.50 ± 0.39	2.25 ± 0.29
25% Legume	5.5 ± 0.77	30.02 ± 0.79	2.57 ± 0.07	307.9 ± 6.57	102.92 ± 9.78	1.75 ±0.92	2.94 ± 0.60
50% Legume	5.5 ± 0.95	27.77 ± 0.37	2.48 ± 0.19	312.9 ± 21.90	114.25 ± 21.33	2.71 ± 0.89	2.43 ± 0.66
75% Legume	5.0 ± 0.05	29.32 ± 0.39	2.45 ± 0.15	328.7 ± 23.50	131.40 ± 26.17	3.75 ± 0.78	2.81 ± 0.61
100% Legume	5.5 ± 0.09	28.74 ± 0.76	2.49 ± 0.12	260.5 ± 28.03	78.16 ± 6.59	1.11 ± 0.34	2.90 ± 0.32
LEG linear (LEG)	F=0.45, *p*=0.50	F=0.93, *p*=0.34	F=0.29, *p*=0.59	F=5.39, ***p*=0.02**	F=0.79, *p*=0.38	F=0.20, *p*=0.66	F=0.49, *p*=0.48
NADD	F=110, ***p*<0.0001**	F=1.4, *p*=0.25	F=16.10, ***p*=0.0002**	F=27724, ***p*<0.0001**	F=1068, **p<0.0001**	F=70.54, ***p*<0.0001**	F=172, ***p*<0.0001**
LEG ^{2} *x* NADD	F=0.01, *p*=0.92	F=2.01, *p*=0.16	F=0.02, *p*=0.87	F=11.98, ***p*=0.001**	F=2.90, p=0.09	F=2.76, *p*=0.15	F=0.15, p=0.70
LEG ^{3} *x* NADD	F=0.01, *p*=0.94	F=4.81, ***p*=0.04**	F=0.59, *p*=0.44	F=0.88, *p*=0.35	F=1.27, *p*=0.27	F=8.45, ***p*=0.006**	F=0.19, *p*=0.66

Some parameters not reported as non-significant.

4.5 Discussion

The aim of this study was to investigate how grassland legume proportion and N addition interact to affect plant-soil properties with feedback consequences for ecosystem GHG emissions. Plant-soil properties and GHG emissions showed significant interactions between legume proportions and N-fertiliser aplication. However, greater diffences were observed under unfertilised and fertilised soils. Below, we will discuss the effect of grass-legume mixture on plant productivcity and shoot N uptake, and how legume proportions affect GHG emissions in the presence or absence of N, linking them with plant productivity and plant-soil properties.

4.5.1 Effect of grass-legume mixtures and N addition on plant productivity and shoot N uptake

Agreeing with our hypothesis, increases in legume proportion in grass-legume mixtures enhanced plant productivity and shoot N uptake, with fertilised soil showing greater effects (Fig. 4.3, 4.4). The increase was non-linear i.e. plant productivity was higher in grass-legume mixtures compared to monocultures (either only grass or only legumes) and many mechanisms can explain these changes.

Leguminous species can significantly affect grassland biomass production (Spehn et al., 2000) due to their ability to fix atmospheric N_2 in the soil providing N rich organic matter (Mulder et al., 2002, Spehn et al., 2002). Studies suggest that mixtures containing legumes produce three times greater biomass compared to mixtures without legumes (Spehn et al., 2000). Besides the increase of N pools in biomass (due N_2 fixation), legumes in a mixture with non-leguminous species can increase plant productivity by spatial or temporal complementarity of resource use in soil, increasing N use efficiency (van Ruijven and Berendse, 2005) and facilitating N transfer from legumes to grasses (Høgh-Jensen and Schjoerring, 1997, Laidlaw et al., 1996).

Grasses and legumes have different functional traits, including leaf/root morphology, which may be responsible for different strategies of resources use (Table 4.1). Clovers (*Trifolium* spp) are known to have thicker, shorter, and less branched root system (Haynes, 1980) i.e. lower SRL, and exhibit weak competition for nutrients with low

mobility in the soil. While *Agrostis* spp are more soil exploitative species, which invest more energy in the development of roots to acquire nutrients from the soil, higher root uptake capacity (Maire et al., 2009), exuding more C compounds than conservative species (De Deyn et al., 2008). The response of mixtures in biomass and productivity may be due to different species functional traits and their priority in investments and complementarity in resource use.

N-use efficiency did not increase in mixtures as shown by shoot CN ratio (Fig. 4.4), but were greater in grass monoculture. This could be explained due to higher investment in stems (especially considering *Agrostis* spp), which have higher CN ratio (Spehn et al., 2000, van Ruijven and Berendse, 2005). The increase of N transfer from legumes to grasses, affecting biomass production (McElroy et al., 2017) is another important mechanism. As shown by the N content in the grass in monoculture and mixtures, N transfer to grasses from legumes in mixtures was not significantly different (Fig. 4.4), suggesting it is not the main mechanism by which N yield was increased in mixtures. N transfer between plants is highly dependent on mineral-N in the soil, environmental stresses, and the quality and quantity of root exudates (McElroy et al., 2017, Paynel et al., 2001).

Shoot N uptake was non-linearly affected by legume proportion (Fig. 4.4), showing that with a higher percentage of legumes, N yield did not continue to increase (Fig. 4.4). Grassland field experiments suggest that most of the benefits on plant productivity of legumes in mixtures are evident at around 30-50% of legume proportion (Suter et al., 2015). This correlates well with our findings, where legume proportions between 25-50% showed higher productivity and N uptake, and were significantly greater than either grass or legume monoculture in both unfertilised an fertilised soils (Fig. 4.3, 4.4). However, there is only a small difference between the three grass-legume mixture treatments. Although legume proportions treatments are described as 25, 50 and 75%, these numbers are based on sown legume abundance (Fig. 4.1), and not legume biomass and this might explain why differences in productivity between the three mixture treatments were not much significant as we expected.

4.5.2 Effects of legume proportion on GHG emissions in unfertilised soils

In unfertilised soils, despite the increase in plant productivity and total N uptake with increasing legume proportion, there was no significant effect of legume proportion on ecosystem respiration or N_2O emissions but there is a significant effect on CH_4 emissions.

In relation to the lack of significant effect on N_2O under N-limiting conditions, it has been suggested that the flux rate through N pools (Jones et al., 2005a) is arguably more important than the actual size of the relative N pools. It has been shown that fast-growing species such as *T. pratense* and *A. capillaris* increase N mineralisation from soil organic matter to a greater extent than more conservative species, with consequent increases in their growth (Personeni and Loiseau, 2005, Van Der Krift and Berendse, 2001). This finding may in part explain the higher biomass production of mixtures of these species in this study. In addition, the higher N availability may temporarily enhance N_2O emissions, given that soil microbes such as nitrifiers and denitrifiers are good competitors for both NH_4^+ and NO_3^- (Hodge et al., 2000). However, as found in many studies, the rate of net N uptake is four- to six-fold higher for faster growing species compared to slower growing plant species (Poorter et al., 1991). This fast flux between soil N supply and N use by both plants and microorganisms would result in no build-up of a soil mineral-N pool (Abalos et al., 2014), agreeing with the results obtained by Jackson et al. (1989) in unfertilised grassland, and would explain the decoupling between biomass production and N_2O emissions. As a consequence, under N-limiting conditions, the acceleration of the N cycle caused by these two fast-growing species (Orwin et al., 2010) is likely to offset the production-induced reduction in N_2O emissions that we hypothesised. In this context, the presence of these two fast-growing species might increase the input of C and N root exudates especially from legumes, which typically stimulate the growth of soil microbes (Denton et al., 1998, Mawdsley and Bardgett, 1997). Increases in microbial biomass may have increased microbial respiration and offset the decrease in ecosystem respiration, and therefore not be affected by increases in legume proportion (Fig. 4.2).

Increases in legume proportion affected CH_4 uptake in both unfertilised and fertilised soils (Fig. 4.2), and legumes and/or grass monocultures did not differ from each other; however, the three grass-legume mixtures showed different patterns (Fig. 4.2). Species

interactions might have altered root densities and architecture, which may lead to change in soil aeration, spatio-temporal organic C deposition, or N use pattern (Niklaus et al., 2016). The increased productivity by mixtures might translate into the supply of organic substrates to soil microbial community, increasing its activity, augmenting oxygen consumption and soil diffusive conductance (Grosso et al., 2000, Smith et al., 2003), these will be in turn determine the CH_4 production from soil (Ball et al., 1999). Additionally, interaction between grasses and legumes might affect the microbial community structure (Suwanwaree and Robertson, 2005), thus changing the biomass and activity of methanotrophs and methanogenic in the soil.

The use of species with contrasting plant traits may affect the N release and uptake by plants (Fig. 4.4), consequently affecting the NH_4^+ concentration in the soil (Table 4.5). Many studies suggest that NH_4^+ may inhibit CH_4 oxidation (Hütsch, 1998, Rime and Niklaus, 2017), however it may not affect if NH_4^+ occur in a different spatial niche active methanotrophs (Hartmann et al., 2011). The effect of NH_4^+ on CH_4 oxidation is related to the competitive enzymatic process between methanotrophy and nitrification in soils (Baggs and Blum, 2004).

The effect on CH_4 emissions can be, therefore, an indirect effect of legume proportions, i.e. related to the interaction between grasses and legumes specifically. It shows that plant diversity can affect CH_4 fluxes, but there are many ecological interactions to consider, thus is difficulty to define only one possible mechanism. In general, the short duration of the experiment, the low level of plant diversity and the lower realised legume proportion (Fig. 4.1) may be contributed to the limited effect on N_2O and CO_2 emissions from the soil.

4.5.3 Effects of legume proportion on GHG emissions in fertilised soils

In fertilised soils, contrary to our hypothesis, N_2O emissions were not affected by increases in legume proportion and it can be largely explained by legume species production. The presence of N in the system was a determining factor for a reduction and/or suppression of legumes biomass (De Deyn et al., 2011, Smith et al., 2008b), and probably to a decrease of N_2 fixation (Ledgard et al., 2001). It may also affect the nodule production or induce a reduction of N fixation, which is shown, by the reduction of N content in legumes species with increasing legume proportion.

The most likely N_2O-forming processes in legume-based cropping are the decomposition of legumes residues after harvest and then from the N release through root exudates during the growing season (Rochette and Janzen, 2005). However, as the experiment was only short-term (40 days) and conducted in a controlled condition, residue decomposition and N release may be negligible, and no effect was found on N_2O emissions from the soil. Many different abiotic and biotic factors may be changing the ecosystem processes, relating indirectly to N_2O emissions. In this study, emissions are dominating by the mineral-N applied as N-fertiliser, and its availability in the soil.

Contrary to our hypothesis, ecosystem respiration was decreased by increases in legume proportion under non-N-limiting conditions. N addition might promote a reduction of legume biomass (favouring grasses) (McElroy et al., 2017) reducing total biomass, which may be the reason for the decrease in ecosystem respiration. Nyfeler et al. (2011) also showed that grasses benefited from N transfer from legumes in fertilised soil (150 kg N ha^{-1}). Additionally, some research suggest that root biomass is reduced in fertiliser treatments compared to no fertiliser (Chirinda et al., 2012), due to higher root exploitation of soil to overcome the low nutrient availability in unfertilised soils. This reduction in root biomass might also decrease root respiration and the overall ecosystem respiration. In addition, the reduction in ecosystem respiration may be related to less energy needed (especially by legumes) to acquire N in grass-legume mixtures (Drinkwater et al., 1998). Although our study did not find significant differences in soil C stock (perhaps due to the short-term of the incubation experiment), other studies have suggested that legumes increase soil C and N stock, reducing nutrient losses to the atmosphere (De Deyn et al., 2011). Li et al. (2016) also suggested that grass-legume ratio of 1:1 increased both total soil C and N at 0-40 cm depth, leading to an increase of use efficiency.

4.5.4 Implications for grassland management and climate change

It is important to highlight that results from incubation experiments need to be used with caution because they are mainly used to investigate mechanistic processes and findings must be evaluated in the field for an accurate result. Furthermore, our observed effects may vary as a function of soil type and moisture content. The use of grass-legume mixture showed to be efficient in increase plant productivity either in unfertilised or fertilised soils. This brings a potential strategy of reducing N-fertiliser

use in the field, an opportunity for climate change mitigation. Besides that, the use of legumes in grass-legume mixtures might also reduce nitrate leaching to soil (Ledgard et al., 2009, Loiseau et al., 2001, Nyfeler et al., 2009). It is also important to determine the adequate proportion of legume in mixtures used in the field without compromising plant production, since legumes in excess would result in a reduction in productivity (Suter et al., 2015). Including legumes in grasslands may deliver equivalent productivity to fertiliser, and although we did not find a reduction in N_2O emissions in this short-term experiment, in the long-term including legumes and reducing N-fertiliser inputs would result in a more closed efficient N cycle with environmental benefits.

4.6 Conclusions

Plant productivity and shoot N uptake showed to be higher in grass-legume mixtures compared to either grass or legume monocultures. This increase in plant productivity showed to have an important effect on ecosystem respiration in grass-legumes mixtures under non-N limiting conditions. In unfertilised and fertilised soils, increases in legume proportion did not affect N_2O emissions from soil, although there is an increase under fertilised soil due to higher soil mineral-N itself rather than legume effect in mixtures. Mixtures with different functional traits suggest a great opportunity to balance C and N cycle, with effects on GHG emissions from soil. Increases in legumes proportion did effect non-linearly CH_4 emissions from soil either with or without N-fertiliser addition. Interactions between grass and legumes seem to be responsible for these changes, affecting soil aeration, spatial-temporal organic C deposition, or N use pattern. The general lack of responses of GHG emissions under increases in legume proportion may be attributed to the short duration of the experiment, the low level of plant diversity and the lower realised legume proportion. Other studies are needed to confirm the findings in this study, although it was a great step to improve our knowledge referring the interaction effect of legume proportion and N addition on N and C cycle in grasslands and its potential effect on climate change.

5 Model predictions of grassland greenhouse gas emissions resulting from interactions between climate and management factors

Arlete S. Barneze [1,2], Mohamed Abdalla [3], Jeanette Whitaker [2], Niall P. McNamara [2] and Nick J. Ostle [1]

[1] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

[2] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK.

[3] Institute of Biological and Environmental Sciences, School of Biological Sciences, University of Aberdeen, 23 St. Machar Drive, Aberdeen, AB24 3UU, UK.

5.1 Abstract

Field management activities and their interactions with climatic variables have significant impacts on greenhouse gas (GHG) emissions from grassland soils. Mathematical models can be used to simulate management and potential changes in climate beyond the temporal extent of shorter-term field experiments. In this study, field measurements of N_2O, CO_2, and CH_4 emissions were used to validate the DNDC (DeNitrification-DeComposition) model. The model was then used to predict changes in GHG emissions with interactions between climate warming and grassland management in a 30-year simulation. Validations suggest that N_2O emissions were well described by N-fertilised treatments, while non-fertilised treatments showed higher variation between measured and simulated values. Ecosystem respiration was well described prior to hay meadow cutting but afterwards emissions were much higher than those simulated. Fluxes of CH_4 were on average negative and largely negligible for both simulated and measured values. Some discrepancies in the model fit to measurements might be improved with further site-specific calibration and parameterisation. The long-term scenario projections suggested that net GHG balance would be positive under all treatments and their interactions. Interactions between warming and N addition showed the most positive impact on GHG emissions from grassland soils. Overall, the results of the modelling confirm that the interactive effects of grassland management and climate warming could increase GHG feedbacks.

Keywords: DNDC model, GHG emissions, grassland, climate change, management.

5.2 Introduction

Grasslands are a major contributor to the exchange of greenhouse gases (GHG) in the biosphere, with their fluxes being intimately linked to prescribed management practices. Depending on the magnitude of GHG exchanges into and out of grassland systems, and considering interactions of climate and management, these systems can either be a net source or sink for GHG's. Therefore, understanding the future trajectory of GHG emissions from grassland soil is important for developing mitigation options in a changing world.

Studies demonstrate considerable variation in temporal and spatial GHG emissions, especially for nitrous oxide (N_2O), from a range of managed systems including grasslands (Imer et al., 2013, Rees et al., 2013). Over the last decade considerable progress has been made using mathematical models to simulate processes responsible for production, consumption and transport of GHG's e.g. DNDC, Daycent, ECOSSE (Abdalla et al., 2016, Abdalla et al., 2009, Fitton et al., 2017). These models can be used to predict GHG emissions from current management practices and to simulate alternative management scenarios such as differing grazing intensity or nutrient management (Smith et al., 2016). Mathematical models can be applied in different ways including at the site-scale to interpolate missing measurements, to extrapolate results from experimental plots spatially and temporally for agriculture GHG inventories, and to look at past and future time periods (Smith et al., 2012). These models include similar components (soil physics, decomposition, plant growth and nitrogen (N) transformations), but in some cases, use different algorithms to represent these key processes.

The DeNitrification DeComposition (DNDC) model is a process-oriented simulation model of soil C and N biogeochemistry at a sub-daily time step, developed to assess N_2O, NO, N_2 and CO_2 emissions from agricultural soils (Li et al., 1992, Li et al., 1994, Li, 2000). It was originally developed in the USA but it has been used to study systems in China (Li et al., 2001), Canada (Smith et al., 2010) and across Europe (Abdalla et al., 2009, Kesik et al., 2006). It has been applied to grassland (Brown et al., 2001, Giltrap et al., 2010, Levy et al., 2007, Saggar et al., 2007a), cropland (Cai et al., 2003,

Li et al., 2007, Li et al., 2017b) and forest ecosystems (Lu et al., 2008, Tang et al., 2006). The model has reasonable data requirements (see Section 5.3.2, Table 5.1) and is suitable for simulations at a range of temporal and spatial scales depending on its configuration (site-specific, Abdalla et al. (2010), Landscape DNDC, Molina-Herrera et al. (2016)).

The aims of this study were: i) to assess the reliability of the DNDC-model for estimating GHG (N_2O, CO_2 and CH_4) fluxes from a temperate grassland under different management and climate warming treatments, including their interactions, using GHG measurements from the Hazelrigg grassland field experiment (as described in Chapter 2); ii) to explore the effects of management, climate and their interactions, on grassland GHG emissions in a 30-year simulation.

5.3 Material and Methods

5.3.1 Model and field experiment description

The latest version of the DNDC model (version 9.5; www.dndc.sr.unh.edu) was tested against the data obtained from the Hazelrigg grassland field experiment (Chapter 2) and then used to predict future scenarios for the interactions between grassland management and climate warming. Briefly, the field experiment was a full-factorial design for evaluating the interactive effects of warming, N addition and cutting on grassland net GHG emissions (section 2.3.2, Chapter 2). Warming was achieved using open-top chambers, which increased air temperatures by on an average of 2 °C. Nitrogen addition was applied at a rate of 100 kg N ha^{-1} y^{-1}, and cutting was carried out when plants reached 5 cm height (total of six times per year).

The DNDC model contains four main sub-models: soil climate, crop growth, decomposition and denitrification (Li et al., 1992, Li, 2000). The soil climate sub-model calculates hourly and daily soil temperature and moisture as water-filled pore space (WFPS). The crop growth sub-model simulates crop biomass accumulation and partitioning; the decomposition sub-model calculates decomposition, nitrification, NH_3 volatilisation and CO_2 production (heterotrophic and autotrophic respiration). The denitrification sub-model tracks the sequential biochemical reduction from nitrate (NO_3) to NO_2^-, NO, N_2O and N_2 based on soil redox potential and dissolved organic C.

5.3.2 DNDC-model initialisation, validation and sensitivity tests

The model was initialised using site-specific features including soil texture, bulk density, pH and soil organic C (SOC) from the Hazelrigg study site. Meteorological parameters including daily temperature (minimum and maximum), daily precipitation and wind speed were obtained from the Hazelrigg Weather Station (www.lancaster.ac.uk/lec/about-us/facilities/hazelrigg-weather-station) for the period from 1977 to 2016. Details of climate and soil property input data for the DNDC model are listed in Table 5.1.

Table 5.1 DNDC model input parameters.

Input parameters	Hazelrigg Grassland
Climate data	
Latitude (degree)	54° 1' N
Yearly maximum of average Daily temperature (°C)	12.8
Yearly minimum of average Daily temperature (°C)	6.7
Yearly accumulated precipitation (mm)	1333
N concentration in rainfall (mg N L^{-1})	2[a]
Atmospheric CO_2 concentrations (ppm)	385*
Annual increase rate of atmospheric CO_2 concentration (ppm y^{-1})	2
Soil properties (0-10 cm)	
Vegetation type	Moist pasture
Soil texture	Clay loam
Bulk density (g cm^{-3})	1.06
Clay fraction	0.41*
Soil pH	5.3
Initial organic C content at surface soil (kg C kg^{-1})	0.038
Harvest	Sheep grazing/ hay cutting
WFPS at field capacity	0.57*
WFPS at wilting point	0.27*

[a] Neal et al. (2004)

* Defaults values

The model was initialised (pre-run) for 30 years under the historic site management i.e. 30 sheep per hectare (James Heath and Brian Davison, personal communication). Following this initialisation step, simulation scenarios were carried out to reflect the management strategy from each experimental treatment (warming, N addition, cutting and interactions) for the field measurement years of 2015 and 2016. The model was run with perennial grassland specified using the vegetation parameters default (e.g. grass yield, root fraction, water demand) in the DNDC model.

The scenario validation of the DNDC model was made with data collected from the full-factorial field experiment described in Chapter 2 over the two growing seasons. The model testing was conducted by: (1) comparing the measured and modelled temporal pattern of N_2O, CO_2 and CH_4 fluxes, and (2) comparing the measured and modelled cumulative GHG fluxes. Seasonal cumulative fluxes were calculated as the sum of daily fluxes divided by the number of measured/modelled days (Cai et al., 2003).

In order to test the general behaviour of the DNDC model a sensitivity analysis was executed (Li et al., 1992). The response of the model and its constituent sub models to a range of model parameters were tested by varying a single parameter whilst fixing others during one cycle of the model. The tested parameters were air temperature, rainfall, initial SOC and N-fertiliser application rate.

5.3.3 Long-term scenarios

In order to project forward over a longer time series than measured in this study, and to test the range of interactions, a climate dataset from Hazelrigg weather station (1977-2016) was obtained. For this projection historic daily air temperature and rainfall were used, assuming no climate change or variation in atmospheric CO_2 concentrations. This component of the work predicted 30-year changes (up to 2047) in GHG (N_2O, CO_2 and CH_4) emissions under management (cutting and N addition) with interactions with climate warming.

5.3.4 Statistical analysis

The DNDC model accuracies and performance were evaluated by calculating the root mean square error (RMSE), relative deviation (RD) and regression coefficient (r^2)

between measured and simulated values. The RMSE measured absolute prediction error as suggested by Smith et al. (1997), but in a quadratic sense, and is, therefore, more sensitive to outliers (Eq. 1). The RD of the simulated flux from measured flux values was calculated by the following Eq. (2).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (Pi - Oi)^2}{n}} \qquad \textit{Equation (1)}$$

$$RD = \frac{(Pi - Oi)}{Oi} x\ 100 \qquad \textit{Equation (2)}$$

Where *Oi* are the observed values, *Pi* are the simulated values, *n* are the total number of observations and *i* the current observation.

5.4 Results

5.4.1 Simulation of GHG (N_2O, CO_2 and CH_4) emissions from the full-factorial field experiment over two growing seasons

Daily N_2O emissions from the field treatments were described by the DNDC-model (Fig. 5.1-5.7 and Fig. A4.1-4.10, Appendix 4) with an r^2 between simulated and measured of 0.39, and RMSE of 3.15. The modelled cumulative emissions showed a better fit (Table 5.2, r^2=87, RMSE=206), although the r^2 has been overestimated due to high noise in the data (lower and higher values), leading to overfitting the modelled values.

The direction of deviation was different between 2015 and 2016, and this is likely because the model under-estimated N_2O fluxes in 2015 when measured emissions were much higher (Table 5.2). The simulation of N_2O with N-fertiliser treatments (100 kg N ha^{-1}) gave a relative deviation from measured data of -48 and 51% for 2015 and 2016, respectively. Emissions from the no-N fertiliser plots were poorly described by the DNDC-model, with relative deviations from the measured ranging from -102% to 65% (Table 5.2). The average relative variation for all fertilised treatments was 2%, while for all non-N fertilised was -26%.

Simulation of ecosystem respiration was consistent with field measurements prior to 200 Julian days but then overestimated after the hay meadow cutting which occurred

on the 195 (14^{th} July 2015) and 185 Julian days (3^{rd} July 2016) (Fig. 5.1-5.7 and Fig. A4.1-4.10, Appendix 4, r^2=0.34, RMSE=6.1). Differences between simulated and measured seasonal emissions for all treatments ranged from -1.68 to 9.83 kg CO_2-C ha^{-1}. Modelling output for CO_2 emissions for all treatments was overestimated by an average of 34 and 28% for the 2015 and 2016 season, respectively. Differences in the temporal pattern of CO_2 effluxes between measured and simulated values were also particularly large after the hay meadow cutting in both years.

Model simulations predicted low or negative CH_4 fluxes, which agree with the experimental measurements (Fig. 5.1-5.7 and Fig. A4.1-4.10, Appendix 4, r^2=0.21, RMSE=3.6) regardless of the treatment effect. Differences between simulated and measured seasonal emissions for all treatments ranged from 6.15 to 2.03 g CH_4-C ha^{-1}. Model output predicted a higher CH_4 sink compared to measured values and the difference between simulated and measured seasonal emissions for all treatments was -2.68 g CH_4-C ha^{-1}. Differences in seasonal CH_4 fluxes were larger under warming treatments.

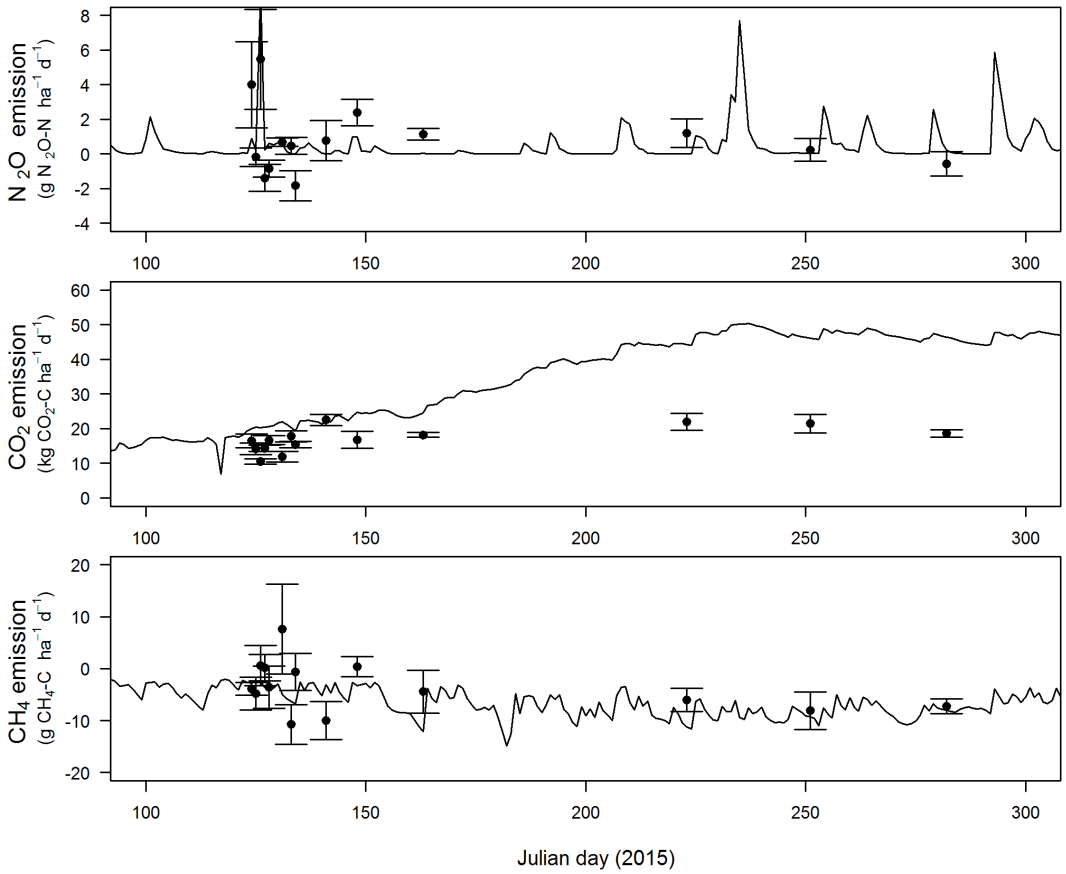

Figure 5.1 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the cutting treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

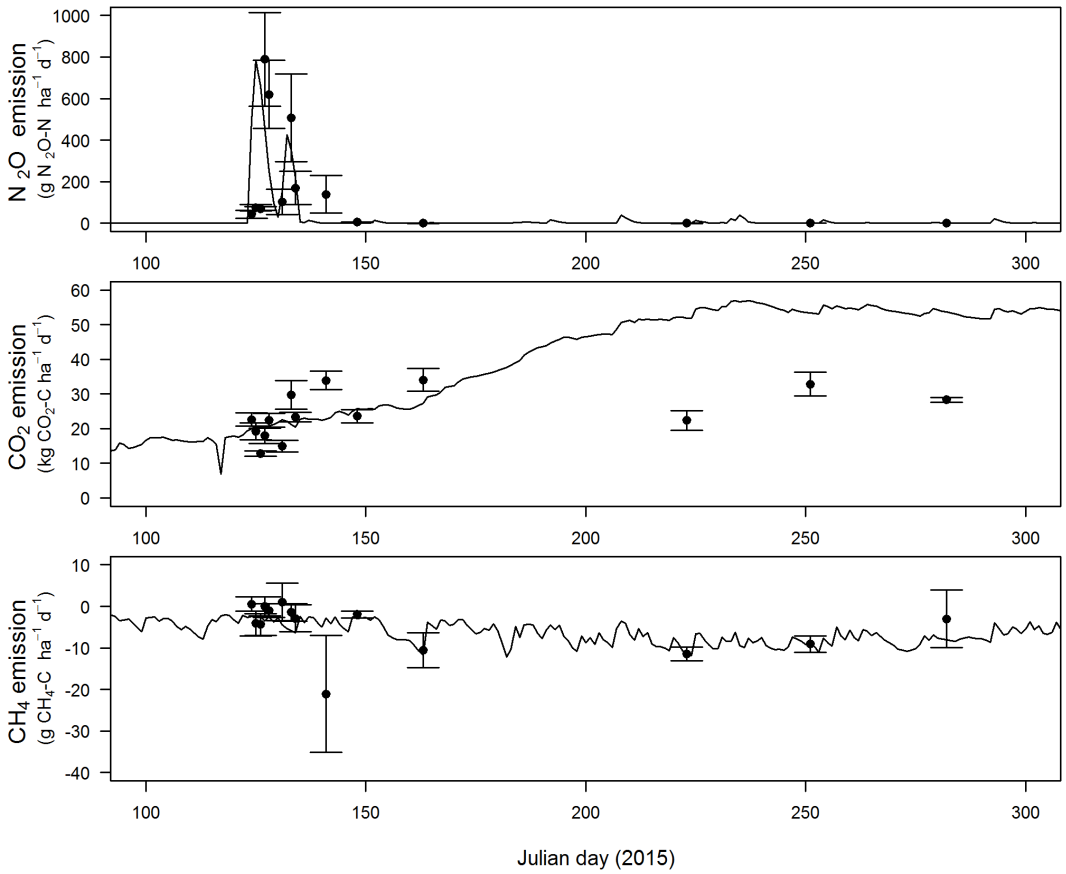

Figure 5.2 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the nitrogen treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

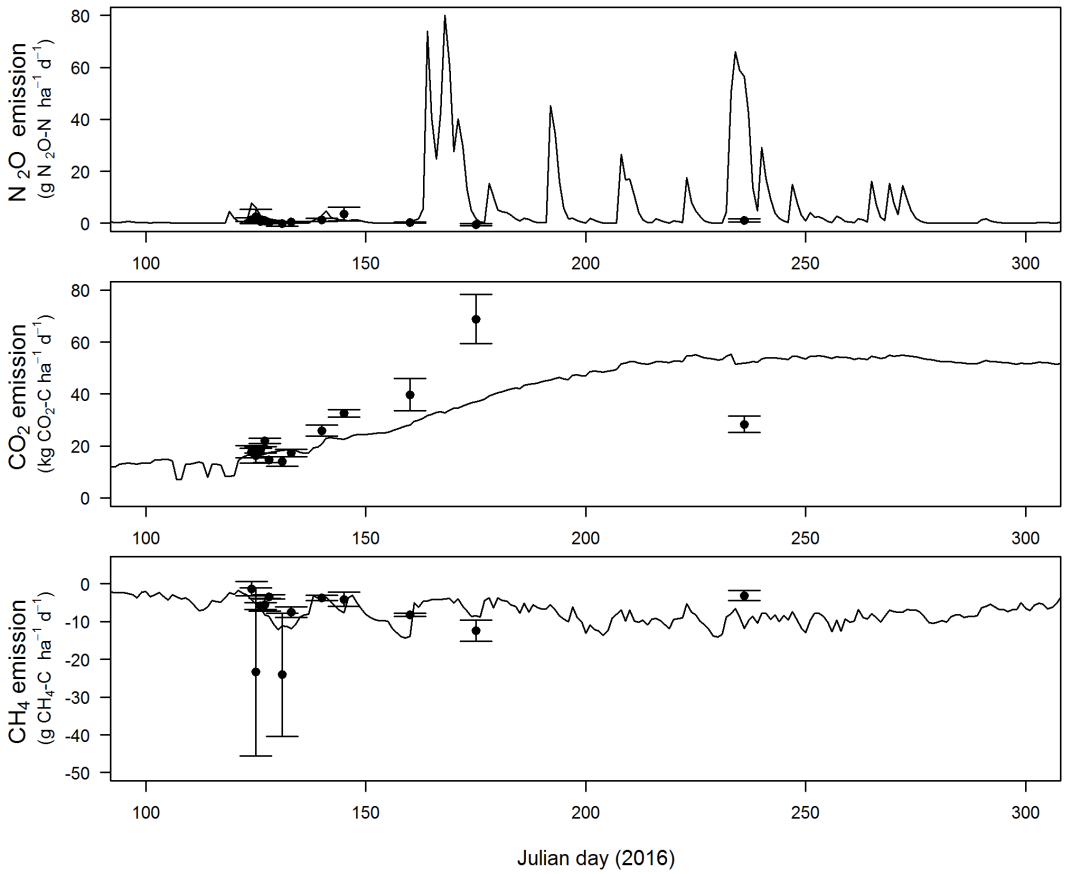

Figure 5.3 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the nitrogen treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

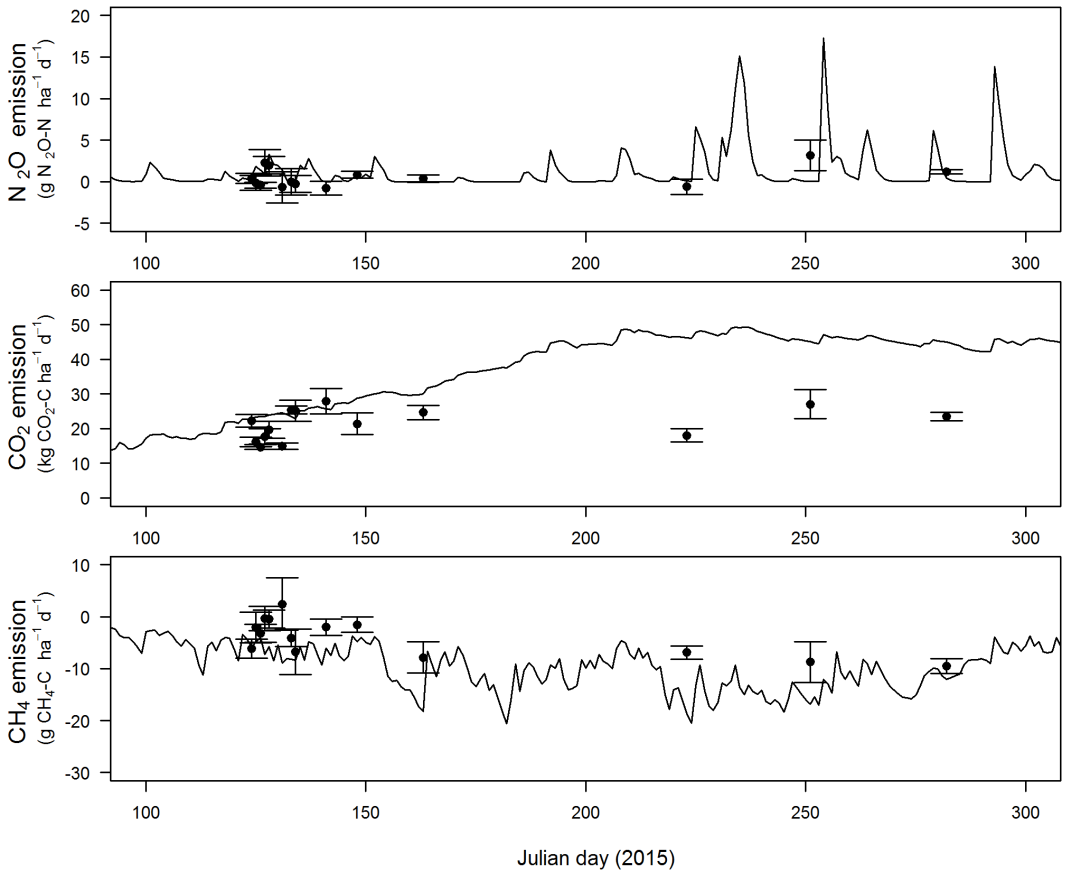

Figure 5.4 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the warming treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

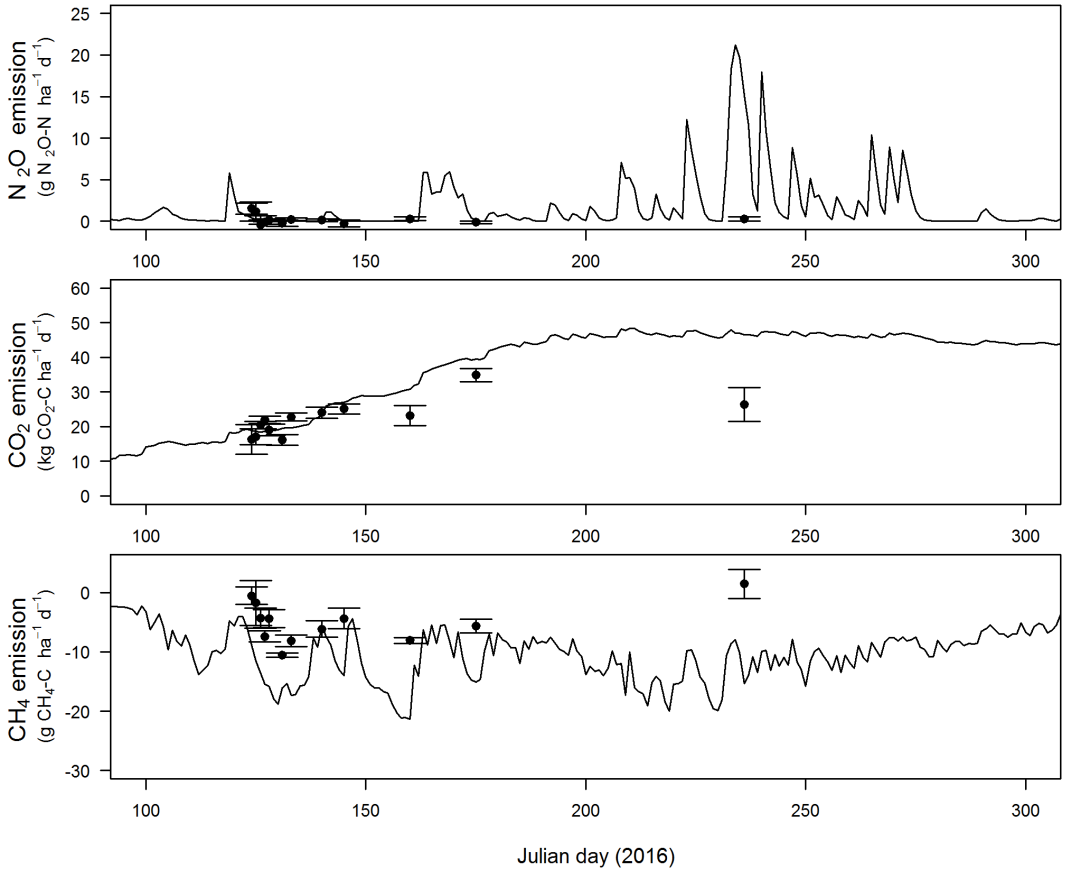

Figure 5.5 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the warming treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

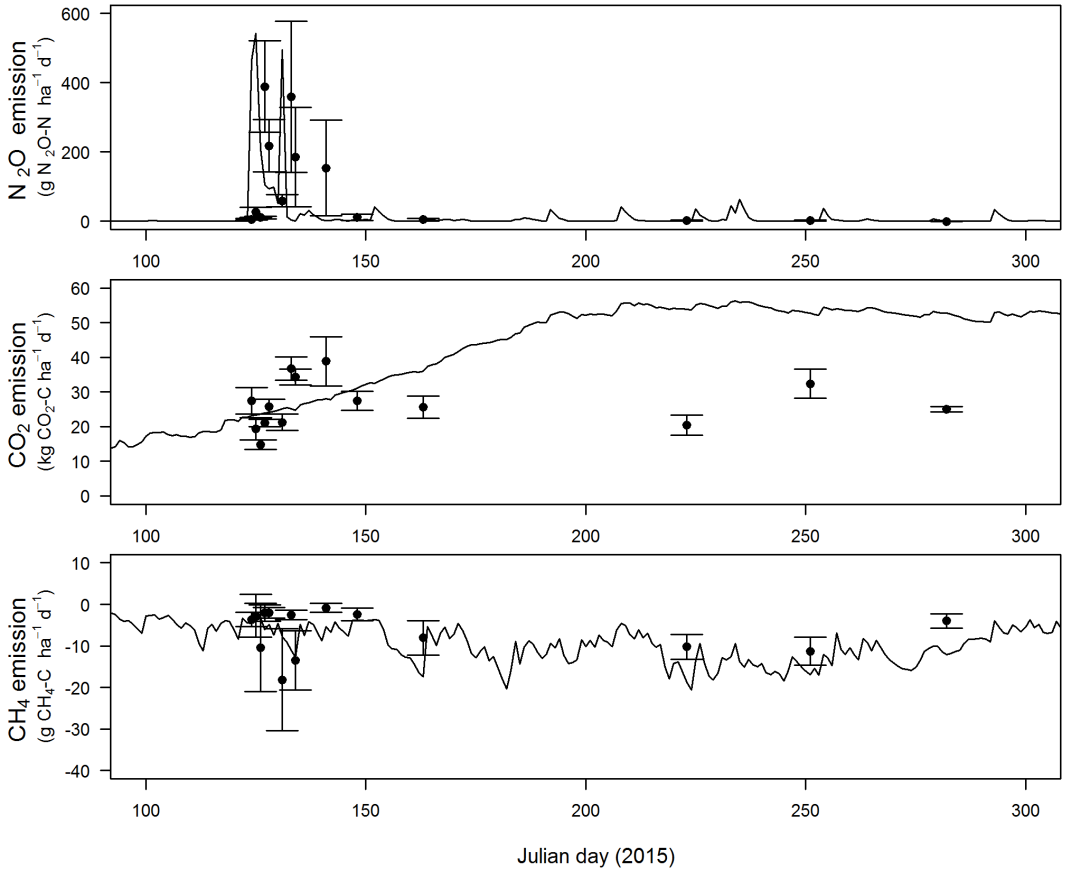

Figure 5.6 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between nitrogen and warming treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

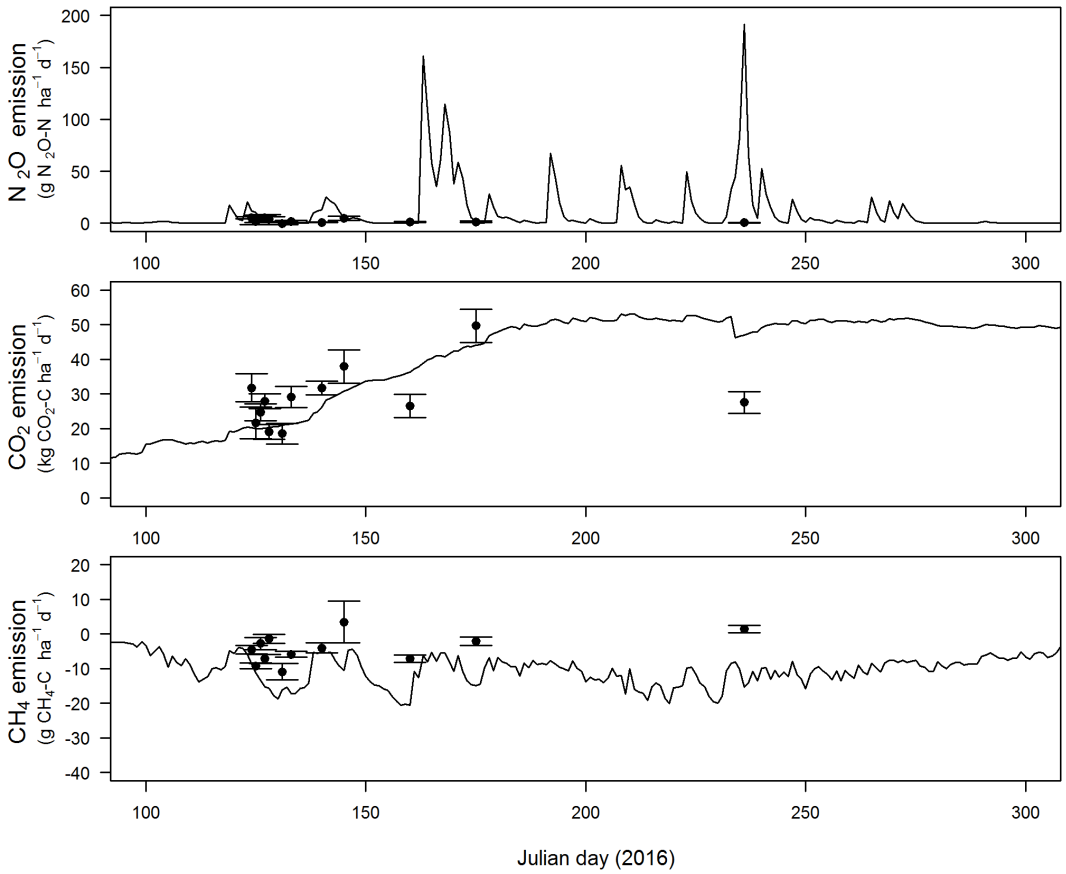

Figure 5.7 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between nitrogen and warming treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

Table 5.2 Measured and DNDC-simulated cumulative N_2O emissions (g N_2O-N ha^{-1}) for the full factorial experiment over two growing seasons.

Treatments	Cumulative N_2O emissions (g N_2O-N ha^{-1})			Deviation between measured and simulated (%)
	Measured	Simulated	Difference	
2015 season				
Control	0.33	0.28	-0.05	-15
Cutting (D)	1.63	0.57	-1.06	-65
Warming (W)	0.32	0.28	-0.03	-11
Nitrogen (N)	252.11	132.38	-119.74	-47
N *x* W	141.67	73.99	-67.68	-48
D *x* N	488.22	245.94	-242.28	-50
D *x* W	0.84	0.92	0.08	10
D *x* N *x* W	245.09	132.82	-112.26	-46
2016 season				
Control	-0.30	0.01	0.31	-102
Cutting	0.01	0.01	0.00	-5
Warming	0.23	0.33	0.10	41
Nitrogen	1.21	1.71	0.50	42
N *x* W	3.04	4.71	1.67	55
D *x* N	5.13	5.84	0.71	14
D *x* W	0.84	0.33	-0.51	-61
D *x* N *x* W	3.34	6.48	3.14	94

Table 5.3 Measured and DNDC-simulated cumulative CO_2 emissions (kg CO_2-C ha^{-1}) for the full factorial experiment over two growing seasons.

Treatments	Cumulative CO_2 emissions (kg CO_2-C ha^{-1})			Deviation between measured and simulated (%)
	Measured	Simulated	Difference	
2015 season				
Control	16.30	22.21	5.91	36
Cutting (D)	15.67	22.21	6.54	42
Warming (W)	20.53	30.36	9.83	48
Nitrogen (N)	22.05	22.90	0.86	4
N *x* W	26.71	27.33	0.62	2
D *x* N	16.35	22.90	6.55	40
D *x* W	16.32	26.00	9.68	59
D *x* N *x* W	19.48	27.33	7.85	40
2016 season				
Control	15.87	18.31	2.44	15
Cutting	11.74	18.31	6.57	56
Warming	20.34	22.99	2.65	13
Nitrogen	19.88	22.99	3.11	16
N *x* W	26.95	25.27	-1.68	-6
D *x* N	15.95	19.83	3.88	24
D *x* W	14.85	22.99	8.14	55
D *x* N *x* W	16.95	25.27	8.32	49

Table 5.4 Measured and DNDC-simulated cumulative CH_4 emissions (g CH_4-C ha^{-1}) for the full factorial experiment over two growing seasons.

Treatments	Cumulative CH_4 emissions (g CH_4-C ha^{-1})			Deviation between measured and simulated (%)
	Measured	Simulated	Difference	
2015 season				
Control	-2.10	-3.70	-1.60	76
Cutting (D)	-2.47	-3.90	-1.43	58
Warming (W)	-2.41	-5.86	-3.45	144
Nitrogen (N)	-3.53	-3.69	-0.17	5
N *x* W	-5.84	-6.21	-0.37	6
D *x* N	-2.13	-3.69	-1.56	73
D *x* W	-0.15	-6.30	-6.15	4155
D *x* N *x* W	-2.17	-5.46	-3.29	152
2016 season				
Control	-6.75	-7.06	-0.30	5
Cutting	-4.06	-6.46	-2.40	59
Warming	-5.30	-11.17	-5.87	111
Nitrogen	-8.79	-6.75	2.03	-23
N *x* W	-4.68	-9.00	-4.32	92
D *x* N	-4.28	-6.75	-2.47	58
D *x* W	-5.53	-11.04	-5.50	99
D *x* N *x* W	-4.13	-10.08	-5.95	144

5.4.2 Sensitivity analysis to GHG emissions

Given the reasonable fit of the model to the treatments, the sensitivities of the model were also investigated. In this procedure, the following parameters were tested:

i) Air temperature
ii) Rainfall
iii) Initial SOC
iv) N-fertiliser application rate

The DNDC model was highly sensitive to changes in these input parameters for predicting N_2O and CO_2 emissions (Fig. 5.8, 5.9); however, it was not sensitive for net CH_4 emission. Increases in air temperature by 3 °C doubled N_2O emission while a decrease of 3 °C reduced emissions by 33%. Changes in rainfall were the most influential parameter (Fig. 5.8) with a 73% increase in N_2O emission when rainfall was increased by 30% and a decrease of 46% when rainfall was reduced by 30%. SOC was also important parameter promoting changes in N_2O emissions. An increase of 30% in SOC doubled N_2O emission, while the same decrease reduced emissions by 40%. An increase of N-fertiliser rate application of 30%, augment N_2O emissions by 46%, however, a decrease of 20 or 30% reduced it only by 26%.

Ecosystem respiration was sensitive to changes in air temperature and SOC but largely invariant with changes to N-fertilisation and rainfall (Fig. 5.9). An increase of 3 °C increased CO_2 emissions by 70%, while a decrease of 3 °C reduced it by 50%. As expected, changes in SOC strongly influenced CO_2 emissions; an increase of 30% in SOC increased emissions by 49%, while the corresponding decrease reduced emissions by 63%. Changes in rainfall did not significantly alter CO_2 emissions; increasing or decreasing rainfall by 30% led to changes in CO_2 emissions by +14% and -12%, respectively. Increases of the N-fertiliser application rate by 30% reduced emissions by 16% and decreasing N-fertiliser application rate by the same amount had a negligible effect.

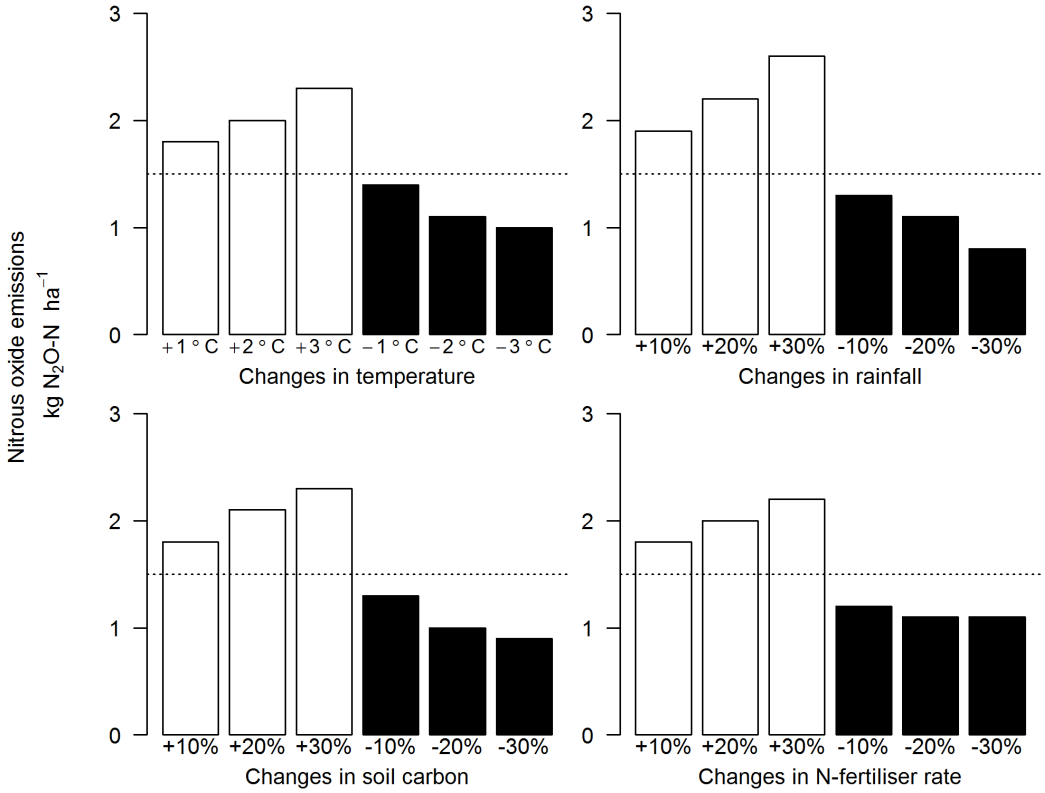

Figure 5.8 Sensitivity of the DNDC-model to changes in climate (temperature and rainfall), in soil characteristics (soil carbon) and in management practice (nitrogen fertiliser rate) on N_2O emissions at the nitrogen treatment at Hazelrigg grassland. Dotted line represents the baseline threshold (annual average maximum temperature 12.8 ºC, average daily precipitation 4 mm, soil carbon 0.0038 kg C kg^{-1} soil, N-fertiliser rate applied 100 kg N ha^{-1}).

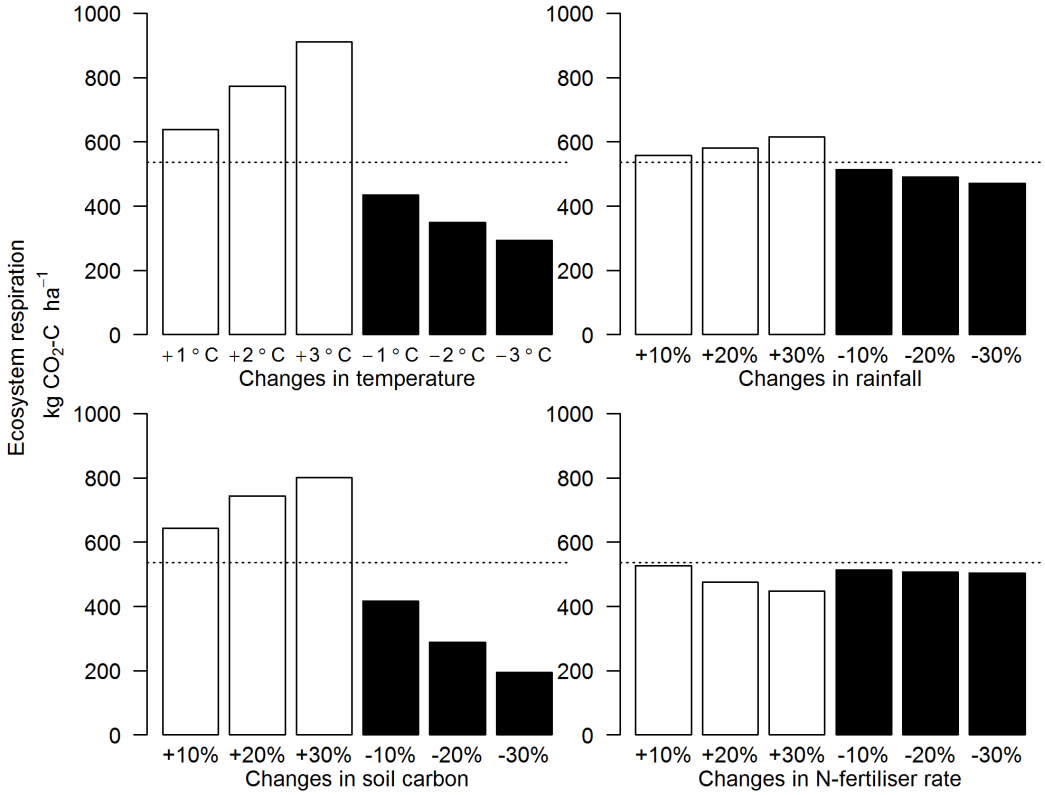

Figure 5.9 Sensitivity of the DNDC-model to changes in climate (temperature and rainfall), in soil characteristics (soil carbon) and in management practice (nitrogen fertiliser rate) on CO_2 emissions at the nitrogen treatment at Hazelrigg grassland. Dotted line represents the baseline threshold (annual average maximum temperature 12.8 ºC, average daily precipitation 4 mm, soil carbon 0.0038 kg C kg^{-1} soil, N-fertiliser rate applied 100 kg N ha^{-1}).

5.4.3 Simulation of GHG changes under field treatments in a long-term scenario

The DNDC model was used to estimate GHG (N_2O, CH_4), SOC and net GHG balance under a long-term 30-year simulation for all field treatments (Table 5.5). Emissions of N_2O and CH_4 were converted to use the concept of global warming potential (GWP) (IPCC, 2007), where the GWP value for CH_4 (based on a 100^{-yr} time horizon) is 34 and for N_2O is 298 (IPCC, 2013). Results showed that under all treatments, except for the warming treatment, the net GHG balance was increased over time (Table 5.5), including all three gases. The warming treatment, with a 2 °C increase in air temperature, decreased the net GHG balance by 15% compared to the control treatment. The effect of N-fertiliser application had the greatest impact on the net GHG balance for the main effect treatments. Changes in the net GHG balance in the cutting treatment were 6% higher when compared to the control treatment. Interactive effects showed greater increases in the net GHG balance compared to singular main effect treatments. The interaction between N addition and warming was increased by 30%, cutting interacted to N addition by 1% and the three-way interaction by 34% compared to the N-fertiliser treatments, while cutting interacted to warming was increased by 25% compared to the control treatment (Table 5.5). Emissions of N_2O were the greatest determinant of changes in net GHG balance, and showed that when N is interacting with climate (warming) or management (cutting) the net emission of N_2O was greater (1.2 and 1.6 t CO_2eq ha^{-1} y^{-1}, respectively, Table 5.5). Similarly for warming (0.161 t CO_2eq ha^{-1} y^{-1}) or cutting (0.119 t CO_2eq ha^{-1} y^{-1}) versus warming or cutting + interaction (0.179 t CO_2eq ha^{-1} y^{-1}).

Table 5.5 Long-term DNDC-estimated (30 years) mean annual GHG (N_2O, CH_4), SOC fluxes and net GHG balance for each field treatment including interactions.

Treatments	N_2O	CH_4	SOC	The net GHG balance
	t CO_2eq ha^{-1} y^{-1}			
Control (C)	0.116	-0.085	0.012	0.043
Cutting (D)	0.119	-0.086	0.012	0.045
Warming (W)	0.161	-0.100	-0.025	0.036
Nitrogen (N)	1.213	-0.082	0.020	1.151
N *x* W	1.597	-0.096	-0.002	1.499
D *x* N	1.222	-0.083	0.020	1.159
D *x* W	0.179	-0.101	-0.025	0.053
D *x* N *x* W	1.639	-0.098	-0.002	1.539

5.5 Discussion

In this work, the reliability of the DNDC-model for estimating N_2O, CO_2 and CH_4 fluxes from grassland was validated against field GHG measurements over two growing seasons (May to October) of 2015 and 2016 (Chapter 2). Two main management practices (N-fertiliser application and cutting) were examined including their interactions with climate warming. Sensitivity analysis of the model was conducted to determine potential impacts on GHG emissions. Finally, using a historic climate (1977-2016) dataset from the Hazelrigg weather station, we explored changes in GHG emissions from the individual and interactive treatments under a longer-term 30-year simulation (up to 2047).

5.5.1 DNDC effectiveness on simulating GHG emissions

Seasonal emissions of N_2O from the N-fertilised treatments were fairly described by the DNDC-model, with differences between measured and modelled values ranging from 3.14 to 242 g N ha^{-1} and with simulated over and underestimated emissions (Table 5.2). The average relative variation between simulated and measured fluxes for the N-fertilised treatments was -2%. Similar deviations using the DNDC model for medium and high N-fertiliser input scenarios have been reported in arable fields by Abdalla et al. (2009) (79 kg N ha^{-1}; 20% deviation and 159 kg N ha^{-1}; 6% deviation) and for grass by Hsieh et al. (2005) (337 kg N ha^{-1}; 33% deviation). The deviation between simulated and measured for the annual N_2O emissions were also high from managed European grasslands being approximately 100% (De Vries et al., 2005). Deviations increase significantly, as fertiliser input is reduced (Abdalla et al., 2009).

In general, the temporal pattern of N_2O emissions was different between simulated and measured data; DNDC extended the influence of added N-fertiliser over a wider time period and produced smaller peaks (e.g. Fig. 5.3, 5.7, more pronounced in 2016). In most cases the model captured N_2O peaks but these often occurred earlier than in the observed (1-2 days before, e.g. Fig. 5.2, Fig. 5.6). This difference in peak times can be explained in part due to the representation of WFPS in the model, which overestimated in some parts of the 2015 growing season. The difference between simulated and measured WFPS was 26% and 15% for 2015 and 2016, respectively. WFPS is a critical determinant of N_2O emissions after N-fertiliser application (Dobbie and Smith, 2001),

as it affects the microbial activity in the soil, changing the aerobic to anaerobic microorganisms activity in the soil. Denitrification is considered the major process of N_2O production from soils (Saggar et al., 2013) with higher WFPS (<70%), while nitrification is the main process when the WFPS decreases to 60% (Ruser et al., 2006). Similar discrepancies between DNDC simulations and field measurements of soil water content were also observed by Abdalla et al. (2009), Kröbel et al. (2010) and Chirinda et al. (2011). Sensitivity analysis of the model highlights the importance of soil moisture in driving N_2O emission with a 20% increase in rainfall approximately doubling the N_2O emission (Fig. 5.8) and most likely associated with the stimulation of denitrification (Butterbach-Bahl et al., 2013). Denitrification is also known to be highly sensitive to changes in temperature, as an increase in 3 °C in air temperature doubled N_2O emission (Fig. 5.8). Increases in temperature can enhance soil respiration and microbial activity, leading to an increase of anaerobic sites, which favour the denitrification process (Butterbach-Bahl et al., 2013). Nitrification is also affected by temperature and has a close relationship with the seasonal variation in soil and air temperature; N_2O emissions have been observed to increase exponentially with increasing soil temperature (Liu et al., 2011a). Overall, changes in soil moisture and temperature have been shown to explain up to 95% of the temporal changes in N_2O emissions from soil (Butterbach-Bahl et al., 2013).

In the non-N fertiliser treatments, the small range of emissions (0.01 to 0.92 g N_2O-N ha^{-1}) was only weakly described by DNDC model (RD from simulated and measured values of -26%). Part of the reason for these observed differences may be associated with the DNDC model not predicting negative fluxes, which may lead to the overestimation of the modelled N_2O flux. These results are similar to other studies where larger relative variation has been observed between simulated and measured fluxes under unfertilised conditions (Abdalla et al., 2009, Rafique et al., 2011b). Nevertheless, on occasions, the model was very effective in simulating smaller N_2O peaks (e.g. Fig. 5.1, cutting treatment).

As discussed in Chapter 2, N-fertiliser treatments were the greatest driving force of N_2O flux from soils and this was reflected in the outputs of the DNDC-model. Annual fluxes increased by 20% when N-fertiliser was increased by 10%, and by 42% when the rate of N-fertiliser was 30% higher (Fig. 5.8). Similarly, N_2O emissions decreased with a

reduction in N-fertiliser application rate (Fig. 5.8). The application of N-fertiliser directly influences the amount of NH_4^+ and NO_3^- available in the soil, reflecting on N_2O emissions from soils. The model was also very sensitive to SOC (Abdalla et al., 2009); with a 20% increment in SOC corresponding to a 40% increase in N_2O emissions (Fig. 5.8). This agrees with findings from Beheydt et al. (2007) who found a difference on average of a 20% increase in N_2O emission between the highest (+15%) and lowest (15%) SOC content. The availability of soil C has a great impact on the activities of microorganisms, consequently affecting cycling and turnover of nutrients and linking to N_2O emissions from soils.

The DNDC model simulated changes in ecosystem respiration (CO_2 emissions) over time and across the field treatments fairly well but overestimated by on average 21% compared to measured data. The simulated and measured CO_2 emissions were higher in the year 2015 compared to 2016. The difference between simulated and measured was 30% in 2015 and 23% in 2016. This might be due to the increase in simulated soil temperature causing consumption of organic matter by microbes, increasing microbial and root activity (Bond-Lamberty and Thomson, 2010). Temperature showed to be highly important for driving changes in CO_2 fluxes (3 °C increase, increased by 70%; Fig. 5.9, Abdalla et al. (2014)) no matter how other input parameters were modified. Likewise, soil moisture was an important driver for CO_2 emissions e.g. a 14% increase in CO_2 flux after a 30% increase in rainfall (Fig. 5.9). It is likely that soil moisture might impact C mineralisation, by providing improved conditions for microbial activities, increasing microbial oxygen consumption and CO_2 production from the soil (Jabro et al., 2008).

Differences between simulated and measured CH_4 fluxes was marginal and consistent with the overall low CH_4 fluxes observed at Hazelrigg for all treatments (Table 5.4). Cumulative CH_4 differences between measured and simulated were particular higher under warming treatments compared to the other treatments (e.g. Fig. 5.4, 5.5). As pointed out previously, a higher simulated soil temperature compared to the measured data may be the reason for the difference in CH_4 uptake in soils. Although there is a lack of correlation between soil temperature and CH_4 uptake, many measurements show that CH_4 oxidation is sensitive to temperature variation (Zhuang et al., 2013). However, the warming treatment simulation did not consider the potential changes in the

measured soil moisture. Therefore, the differences between simulated and measured CH_4 uptake might be due to the indirect effect of warming (decreases in soil water content).

5.5.2 Long-term effect of the interactions between grassland management and climate warming

Whist DNDC showed some limitations by over- or underestimating absolute values of GHG fluxes under the various treatments it is still a very useful tool for exploring scenarios. In this context, the model was used to estimate longer-term effects of the treatments on GHG emissions over 30 years into the future. The net GHG balance estimated for Hazelrigg grassland soils range between 0.04 to 1.5 t CO_2*eq* ha^{-1} y^{-1} from all the field treatments (Table 5.5). Extrapolated to the UK grassland cover area, this is equivalent to a CO_2 source of 7.2 Mt CO_2*eq* y^{-1}, i.e., 5% of the UK energy supply emissions based on 2015 estimation (BEIS, 2017). It is important to note that the net GHG balance estimation is not taking into account the effect of grazing animals, so it may be an underestimate. Grazing animals are estimated to emit 24 and 3.5 Mt CO_2*eq* (BEIS, 2017) via enteric fermentation and deposition of urine and faeces to the soil, respectively. The N_2O emissions estimated for the long-term simulation (0.11 t CO_2*eq* ha^{-1} y^{-1}) are similar to other grasslands soils in Europe (0.14 t CO_2*eq*. ha^{-1} y^{-1}, Soussana et al. (2007)) and in the UK (0.13 t CO_2*eq* ha^{-1} y^{-1} , Levy et al. (2007)).

Warming effect per se decreased the net GHG balance by 15% in 2047, contradicting studies which suggest that an increase in air temperature would increase CO_2 emissions (Emmett et al., 2004, Fang and Moncrieff, 2001), although studies indicate a reduction in above-ground productivity over nine years of warming (Wu et al., 2012). However, there are a large of uncertainties around this result, as the simulation is not accounting for differences in rainfall events and consequently to changes in soil moisture under warming treatments which might have a larger impact on N_2O emissions (Bai et al., 2013). Further, recent studies (Crowther and Bradford, 2013, Kirschbaum, 2004, Yuste et al., 2010) demonstrate an acclimation of ecosystems whereby microbes over-ride the increase in temperature, limiting substrate mineralisation and consequently soil respiration. The SOC fluxes changed from source to sink under warming treatments and including with interactions.

The effect of N treatment showed a greater increase in the net GHG balance over 30 years of simulation compared to control plots and especially in relation to N_2O emissions, agreeing with findings of Hsieh et al. (2005). Similar results was also found by Saggar et al. (2007a) who showed that N_2O remained elevated in N-fertilised treatments for 10 years, even ceasing N-fertiliser application. The simulations indicate then that long-term N-fertiliser application have a greater impact on N_2O emissions compared to a short-term effect (Schmid et al., 2001). The N_2O fluxes have a threshold response to N i.e. the amount of N lost to the atmosphere depends on the N uptake by plants (McSwiney and Robertson, 2005).

Interestingly, the increase of cuttings per year did not show a great long-term effect on the net GHG balance (+6%, Table 5.5), although observed changes were found for ecosystem respiration and N_2O emissions from the field measurements (Chapter 2). Similarly, LeCain et al. (2002) did not find any changes in photosynthetic, soil respiration and net CO_2 exchange rates in grazed compared to no-grazed pastures. Study from Kang et al. (2013) found in a 30-year simulation a reduction of 17% in ecosystem respiration with moderate grazing. The authors related this to a reduction of above-ground litter input directly affecting soil respiration (decreased by 34%). Although mowing or grazing would have a pronounced impact at a large temporal-spatial scale, Li et al. (2014) did not find that these were sensitivity parameters for soil N storage. Grazing could act as a net source in a 29-year simulation in China, according to Han et al. (2014). Therefore, the effect of cutting/grazing on long-term carbon dynamics is still a gap in knowledge as well as its interactions with future climate.

Although seasonal significant differences were found in the interactions between management and climate (N addition *x* warming, and cutting *x* N addition) in Chapter 2 over 2015 and 2016, longer-term simulations showed that overall differences were higher compared to singular treatments (in particular for the N treatments). Interaction effects, mainly relating the N-fertiliser application and the increase of air temperature, showed greater impacts in N_2O, CH_4 and SOC change from grassland soils (Table 5.5). This is an important outcome as it reflects the real-world scenarios where many drivers co-occur at the same time. The impacts on GHG balance leads us to think on the mitigation options for the future climate change scenarios. Studies from IPCC suggested different climate scenarios where it takes an account the industrialisation and

population growth, determining temperature-sensitive scenarios namely as low, medium, and high (IPCC, 2000). It would be very useful to use these scenarios to predict the effect of these managements (cutting and N-fertiliser) with respect to changes in temperature and rainfall events. Studies have demonstrated that there are small differences between these scenarios (i.e. low and high) in relation to GHG emissions, however they can vary about 3 to 17% compared to baseline scenarios (Abdalla et al., 2014, Abdalla et al., 2010, Gulzari et al., 2017) where no future climate is analysed. In conclusion, these models offer a means to compare different climate scenarios under different grassland management. Nevertheless, interpretation should be cautious as the models still need to be improved, especially calibration of the crop module to site-specific features. Saggar et al. (2007a) also point out dramatic changes in simulations in the first 10 years before model stabilisation. It has been suggested that DNDC still requires further refinement, in particular refereeing to long-term farm-managements effects.

5.6 Conclusions

The DNDC-model was able to estimate GHG fluxes from the grassland field experiment, although revealed some divergences regarding different treatments and over two growing seasons. The discrepancies indicate limitations to the model and a need for calibration and parametrisation for specific conditions in order to determine its suitability in providing reasonable estimates, e.g. using real values where the default ones have been used. Nevertheless, it is important to consider that there is a great variability in measured GHG emissions from soil, which also needs to be considered when comparing modelling results (Ambus and Christensen, 1995, Schelde et al., 2012, Smith et al., 2016, Wei et al., 2015). Changes in air temperature, rainfall, N-fertiliser rates and SOC seems to be sensitive parameters of changes mainly in relation to N_2O and CO_2 emissions. Longer-term scenarios showed that interactions between climate warming and grassland management were highly affected compared to a single drivers of change. Overall results show that interactions between grassland management and climate warming are likely to increase GHG emissions making the need to define mitigation options. Further studies could be done to evaluate the impact of different N-fertiliser application rate and timing, varying cutting frequency and its interaction with climate to choose potential mitigation options for future climate change scenarios.

6 Overall discussion

It is estimated that human population will grow by two to three billion within the next few decades (Lutz and KC, 2010), meaning that intensification of agricultural land for food and fuel will increase. Climate change and global warming will further emphasise these effects, as the global temperature is expected to increase 1.5-2 °C by the end of the century (IPCC, 2014). There is an urgent concern about the combined impacts of agricultural intensification and global warming on carbon (C) and nitrogen (N) cycling in agricultural ecosystems including grasslands, with consequences for soil C sequestration and greenhouse gas emissions (GHG). Grasslands cover 20-40% of the terrestrial land surface and supply food, fuel and fibre to 7.6 billion people (FAO, 2015). It is, therefore, important to find ways to mitigate increased agricultural GHG emissions in the face of predicted changes. However, there is still much uncertainty about the impact of interactions between climate and management on biogeochemical cycling in grassland ecosystems (Arneth et al., 2010).

The overarching aim of this thesis was to improve understanding of the interactive effects of grassland management and climate warming on plant productivity, plant-soil properties, C and N cycling and GHG emissions. Additionally, to determine the impact of plant community manipulations under different nutrient availabilities on C and N cycling. Finally, to examine the interactive effect of climate warming and grassland management on longer-term GHG balances using a mathematical modelling approach. This chapter discusses the main findings of this thesis, their implications and the potential for future research.

There are five key findings from this study. First, warming affected GHG emissions and plant productivity differently. Second, interactions between climate warming and grassland management affected GHG emissions. Third, plant-root traits and plant-soil properties were strong determinants of changes in GHG emissions. Fourth, N_2O and CH_4 fluxes were less affected by the presence of roots and/or mycorrhiza fungi than CO_2 fluxes. Fifth, C and N cycling and GHG emissions were altered by changes in plant proportions in mixtures and under different nutrient availability.

6.1 Warming, GHG emissions and plant productivity

Climate warming has the potential to enhance nutrient mineralisation and nitrification (Bai et al., 2013) and alter the length of the growing season (Post et al., 2009), directly affecting plant growth. Warming can also stimulate microbial and plants metabolism and increase GHG emissions to the atmosphere. In this study, a warming effect was observed on ecosystem respiration, enhancing CO_2 emissions. However, changes associated with plant productivity varied between years. First, warming increased above-ground biomass (AGB) and decreased below-ground biomass. After two years of experimental treatments, warming also decreased N_2O emissions and increased CH_4 uptake (Chapter 2) while decreasing basal soil respiration (Chapter 3).

Some studies have found that the length of warming can cause an acclimation and different ecosystem functions can be altered (Kirschbaum, 2004). Warming may affect root respiration indirectly due to the reduction of soil moisture reducing root and microbial activity, and substrate limitation (Chen et al., 2016a, Luo et al., 2001, Rustad and Fernandez, 1998). Other studies are required to determine the real effect of warming in a multiyear experiment as changes to soil C pools may take longer to occur whereas changes in the activity and composition of soil microbes and roots can occur more rapidly.

To simulate global warming, open-top chambers (OTCs) (Marion et al., 1997) were used in this study (Chapter 2, 3). OTCs are widely utilised in experimental warming studies and can increase air and soil temperature although they may have an indirect effect on soil moisture (Brzostek et al., 2012). In this study, these chambers increased air temperature by 2 ºC which is expected by the end of the century according to the latest report from IPCC (2014). Soil temperature was increased by 0.5 ºC and soil moisture reduced by 18%. Although OTCs may have confounding effects on microclimatic variables, it is still a valid and useful method for a passive increase of air temperature in grasslands, and has been used in many studies (Walker et al., 2016, Ward et al., 2013, Zhang et al., 2015a).

6.2 Interactions between climate warming and grassland management affected GHG emissions.

This thesis confirms the need to study multi-factor rather than single drivers to determine real scenarios for future global changes. Interactions between climate warming and grassland management (i.e. N addition and AGB removal) were found in this study, varied among years and background climate conditions (Chapter 2), and were increased in long-term projections (Chapter 5). Interactive effects changed plant productivity, plant-soil properties, C and N cycling and GHG emissions (Chapter 2).

In the first year of the field experiment, warming interacted with N addition increasing ecosystem respiration, and decreasing N_2O emissions from the soil. During the second experimental year, these interactive effects were not observed, although a decrease of root rhizosphere respiration (Chapter 3) was found. Differences found between growing seasons can be related to changes in microclimate and nutrient availability (Chapter 2). Changes in N_2O emissions over years has been shown to relate to differences in soil moisture and nutrient availability (Butterbach-Bahl et al., 2013, Ussiri and Lal, 2012). The reduction of soil moisture may affect N transformation in the soil, leading to higher N uptake rates by plant roots (Chapter 2). Likewise, warming and N addition may reduce microbial biomass (Graham et al., 2014) affecting the activity of soil nitrifiers and denitrifiers.

The effect of AGB removal and N addition shows a synergistic interaction, increasing N_2O emissions from soil, and antagonistically reducing ecosystem respiration over both study years. Studies show that clipping or grazing promotes a reduction of canopy photosynthesis, slowing the translocation of C to the rhizosphere, ultimately affecting ecosystem respiration. However, the intensity and duration of this effect determines the relative effect on GHG emissions. The simulation of clipping also accelerates root exudation production, liberating nutrients to the soil, increasing for instance mineral-N availability. Although, clipping may result in C limitation to the system, higher soil N concentration promotes an increase of N_2O emissions from soil, due to an increase of microbial substrates for nitrification and denitrification. These effects are only observed at the ecosystem level (Chapter 2), showing no effect on each below-ground component when examined separately (root, mycorrhiza fungi, microbes) (Chapter 3).

Warming interactions with AGB removal did not affect soil or ecosystem GHG emissions or soil properties. However, this interaction may promote changes in C and N proportion in soil and plants mainly in the first year associated with changes of microclimate conditions (Chapter 2). Although soil temperature was increased by the interaction in the two years, this effect may be more evident in the first year due to the difference in soil moisture between years. In addition, the effect of clipping on soil temperature could also be important, as it increased soil temperature (Dijkstra et al., 2012, Luo et al., 2010), which may affect C and N cycling by microbes. Additionally, soil N availability may be increased by cutting (Hamilton and Frank, 2001), and mineralisation stimulated by warming (Rustad et al., 2001), leading to increased N uptake by plants and N limitation in the soil (as shown by an increase of specific root length, Ostonen et al. (2007)). Nevertheless, C and N changes in the ecosystem did not affect GHG emissions overall. Similarly, at the below-ground compartment level (Chapter 3), cutting might reduce canopy photosynthesis, slowing the translocation of C to the rhizosphere (as shown by a reduction of total soil C and N), reflecting the lack of effect on microbial activity and at the ecosystem overall. The interactive effect of warming and AGB removal on GHG emissions will be determined by the balance of AGB removal and labile C input to the soil (Cao et al., 2004, Dijkstra et al., 2012, Raiesi and Asadi, 2006).

Modelling approaches were used to estimate changes in GHG balance under different grassland management and climate warming scenarios in a 30-year simulation (Chapter 5). The DNDC model is a reliable model which already showed reasonable estimation in grasslands and in the UK (Brown et al., 2002, Levy et al., 2007). Although further model parameterisation (in particular for crop growth) is required, the results showed a good estimate of CO_2, N_2O and CH_4 changes (Chapter 2 and 5). The DNDC model was very sensitive to changes in air temperature, soil moisture, soil organic C and N-fertiliser application rate for CO_2 and N_2O, with no changes in relation to CH_4. Long-term scenarios showed greater impacts on GHG emissions under interactions compared to singular drivers of change. These findings showed the importance to study interactions between management and climate, which will help to predict mitigations options for this changing world.

6.3 Root traits were strong determinants of changes in GHG emissions

Trait-based approaches are used widely by ecologists to characterise plant strategies for nutrient acquisition and plant growth rates. Root traits can reflect community dynamics and ecosystem processes (De Deyn et al., 2008, Lavorel et al., 2013) such as GHG emissions from the soil (Abalos et al., 2018). In our study, root traits were highly correlated to changes in C and N cycling in temperate grasslands (Chapter 2), as it reflects the economic aspects of the plants due to the C and N availability in the ecosystem (Eissenstat et al., 2000, Kuzyakov and Bol, 2006). Changes in CO_2, N_2O and CH_4 emissions were related to root traits such as N content; specific root length and root dry matter content explaining around 5% of the variation (Chapter 2). For instance, Ostonen et al. (2007) suggested that specific root length can be used as an indicator of environmental change when nutrient availability is manipulated. Root traits offer an alternative to predict GHG fluxes, due to their correlation; however, further studies are required to determine the potential to use traits to estimate ecosystem functional changes in a variety of climatic conditions and ecosystems.

6.4 N_2O and CH_4 fluxes were less affected than CO_2 emissions by different soil below-ground compartments.

In our study, N_2O fluxes were not affected by the presence or absence of roots and/or fungal mycelium (Chapter 3). As reported in other studies (Hodge et al., 2010, Lazcano et al., 2014), the direct effect of the soil below-ground component on N_2O emissions was weak, although indirect effects of changes in soil moisture and N availability are likely. Soil NO_3^- was decreased in the presence of root and/or mycelium but did not affect N releases to the atmosphere. This can be explained by the not optimum microclimate conditions (WFPS below 40%) to promote N transformation in the soil (Ussiri and Lal, 2012).

Additionally, the presence of arbuscular mycorrhiza (especially in grasslands, Johnson et al. 2001) may also affect indirectly CH_4 uptake from soil, via changes in soil structure (soil aggregation), water retention (Rillig and Mummey, 2006) and C, N and P status of soils. These can be the possible indirect effects reducing CH_4 uptake in the presence of root and/or mycelium in the soil (Chapter 3). Furthermore, other factors may be influencing this effect such as soil pH, C and O_2 availability (Hodge et al., 2010).

The consequence of these results is that N_2O and CH_4 are less affected by root and mycorrhiza than expected. Other studies are required with longer experimental duration, other drivers, and higher resolution GHG measurements over time.

6.5 C and N cycling and GHG emissions were altered by changes in plant proportions in mixtures and under different nutrient availability

Plant productivity had an important effect on soil GHG emissions in grass-legume mixtures, especially under non-N limiting conditions (Chapter 4). Grass-legume mixtures increased plant productivity and shoot N uptake due to the presence of legumes (which can increase biomass three-fold, Spehn et al. (2000), Chapter 4). This increase can be probably related to enhance spatial and temporal use of soil resources (different functional traits, van Ruijven and Berendse (2005)), and due to augment the nutrient transfer between legumes and grasses in mixtures (Høgh-Jensen and Schjoerring, 1997, Laidlaw et al., 1996).

In unfertilised grassland soils, the increase of legume proportion increased biomass production and shoot N uptake; however, it did not reduce N_2O emissions and did not increase ecosystem respiration as we expected (Chapter 4). This can be explained by the higher mineralisation of N from soil organic matter by fast-growing species (*T. pratense* and *A. capillaris)*, increasing plant growth. In addition, the acceleration of the N cycle caused by these two fast-growing species (Orwin et al., 2010) is likely to offset the production-induced reduction in N_2O emissions that was hypothesised. Similarly, the presence of these two fast-growing species might increase the input of C and N root exudates stimulating microbial biomass (Denton et al., 1998, Mawdsley and Bardgett, 1997) and microbial respiration, acting to offset the total respiration (Chapter 4).

In fertilised soils, when N availability was higher, the effect of an increase in legume proportion on N_2O emissions was highly related to the availability of mineral-N in the soil. First, N addition is related to a suppressing of legumes and its biomass (De Deyn et al., 2011, Smith et al., 2008b) and N_2-fixation (Ledgard et al., 2001), leaving N content in the soil. The quantity of N applied and not used by the grass-legume mixtures will probably determine the N_2O emissions from the soil. Ecosystem respiration was decreased by increases in legume proportion under non-N limiting conditions, and the reason may be due to the increase plant productivity in grass-legume mixtures.

This study suggests that in unfertilised soils, legume species (fast-growing species) may be combined with a conservative species to slow down the N and C cycles, thereby reducing N_2O emissions and enhancing soil C sequestration. Conversely, in fertilised soils, fast-growing species might be combined as the effect on N_2O emissions is highly influenced by changes in soil mineral-N, and it promoted a reduction in ecosystem respiration overall. Other studies are needed to confirm the findings in this study, such as field experiments with a variety of N addition treatments over a range of different environmental conditions on grassland ecosystems. However, it was a step to improve knowledge regarding legume proportion and N and C cycling in grasslands.

6.6 Conclusion

The interactive effects of climate warming and grassland management can have different impacts on C and N cycling in grassland plants and soils. This thesis highlights that singular management drivers alter C and N cycling and GHG emissions, but that effect direction might be changed by the interaction with climate warming and/or other management strategy. Field measurements in nutrient cycling generally depend on microclimatic conditions, plant productivity (above- and below-ground) and soil properties, altering the short-term effect. However, modelling simulations showed that over longer-term there will be interactions between climate warming and grassland management, with potential feedbacks to future climate change. Although roots, mycorrhizal fungi and microbes are important components of nutrients cycling, when examined separately, their contribution to respiration was generally resistant to changes to climate warming and management. The release of C and N as GHG emissions to the atmosphere was mainly related to changes in soil structure by the below-ground components in grassland soils. Plant C and N cycling responses to different grass-legume proportions differed depending on the nutrient availability in grassland soils. The response of grassland biogeochemical cycles to species proportions are related to differences in plant functional traits, which may determine the plant strategy to acquire nutrients from soil. The research offers insight into potential means by which grassland management could be used to mitigate GHG emissions, depending on N availability. Overall, this thesis shows some of the mechanisms by which interactions between climate warming and grassland management may alter C and N cycling with feedbacks to GHG emissions, and to determine the real impact over the future climate change.

6.7 Future research

This thesis has investigated changes in C and N cycling under interactions between climate warming and grassland management, and under interactions between plant species proportions and nutrient availability. This study raises key questions, which could be addressed through further research.

First, future predictions of climate change estimate that besides changes in air temperature, there will be alteration of rainfall events and an increase of CO_2 concentration in the atmosphere. Studies integrating changes in temperature, soil moisture and CO_2 concentration would give a more accurate prediction of GHG balance and the interaction with grassland management. This could be done, for instance, by experimental studies and/or using modelling approaches with the inclusion of IPCC scenarios for future climate change.

Second, a recent and simple method was used to partition the below-ground component respiration, and the impact of the presence or absence of roots and/or mycorrhiza fungi on N_2O and CH_4 emissions from grasslands, but with no clear effects on emissions. Field experiments could be done for longer-time periods to find out the impact of each below-ground component on the C and N cycling. Further insight could be gained from the evaluation of the respiration contribution of each below-ground component and its correlation with mycorrhiza and microbial biomass. Determining then if the interactive effect influence biomass and/or nutrient cycling below-ground. Additionally, soil temperature and moisture should be taken in each below-ground component to determine, for instance, the temperature response of the component respiration flux, and microclimate influence on the nutrient cycling.

Third, this thesis evaluates the plant composition effects on GHG emissions from grassland soil using one grass and one legume species in differed plant proportions. Given the importance of plant composition in defining gas exchange between soil and the atmosphere, and its importance on soil properties, further studies could extend the number of species in plant composition. An increase of plant composition would therefore improve our knowledge about plant functional trait and ecosystem functions. Additionally, it would provide an opportunity to define mitigation options, by

increasing plant composition of specific species and diminishing nitrogen application in grassland soils.

Fourth, the modelling chapter of this thesis estimates the GHG change under interaction between climate warming and grassland management. Besides showing a reasonable estimation compared to the field measurements, model parameterisation is required to comprise crop features and conditions at the specific experimental site. As warming was included in the model simulation, other changes as rainfall and CO_2 concentration should be included to have an accurately long-term scenario. Additionally, comparison with other models, e.g. Daily Daycent, could be done to evaluate the effectiveness of the models in predict GHG emissions from soils.

References

ABALOS, D., DE DEYN, G. B., KUYPER, T. W. & VAN GROENIGEN, J. W. 2014. Plant species identity surpasses species richness as a key driver of N(2)O emissions from grassland. *Glob Chang Biol,* 20**,** 265-75.

ABALOS, D., VAN GROENIGEN, J. W. & DE DEYN, G. B. 2018. What plant functional traits can reduce nitrous oxide emissions from intensively managed grasslands? *Global Change Biology,* 24**,** e248-e258.

ABDALLA, M., HASTINGS, A., HELMY, M., PRESCHER, A., OSBORNE, B., LANIGAN, G., FORRISTAL, D., KILLI, D., MARATHA, P., WILLIAMS, M., RUEANGRITSARAKUL, K., SMITH, P., NOLAN, P. & JONES, M. B. 2014. Assessing the combined use of reduced tillage and cover crops for mitigating greenhouse gas emissions from arable ecosystem. *Geoderma,* 223-225**,** 9-20.

ABDALLA, M., JONES, M. & WILLIAMS, M. 2010. Simulation of N2O fluxes from Irish arable soils: effect of climate change and management. *Biology and Fertility of Soils,* 46**,** 247-260.

ABDALLA, M., RICHARDS, M., POGSON, M., SMITH, J. U. & SMITH, P. 2016. Estimating the effect of nitrogen fertilizer on the greenhouse gas balance of soils in Wales under current and future climate. *Regional Environmental Change,* 16**,** 2357-2368.

ABDALLA, M., WATTENBACH, M., SMITH, P., AMBUS, P., JONES, M. & WILLIAMS, M. 2009. Application of the DNDC model to predict emissions of N2O from Irish agriculture. *Geoderma,* 151**,** 327-337.

ALBERTON, O., KUYPER, T. W. & GORISSEN, A. 2005. Taking mycocentrism seriously: mycorrhizal fungal and plant responses to elevated CO2. *New Phytologist,* 167**,** 859-868.

ALLISON, S. D., GARTNER, T. B., MACK, M. C., MCGUIRE, K. & TRESEDER, K. 2010. Nitrogen alters carbon dynamics during early succession in boreal forest. *Soil Biology & Biochemistry,* 42**,** 1157-1164.

AMBUS, P. & CHRISTENSEN, S. 1995. Spatial and Seasonal Nitrous-Oxide and Methane Fluxes in Danish Forest-Ecosystems, Grassland-Ecosystems, and Agroecosystems. *Journal of Environmental Quality,* 24**,** 993-1001.

AMBUS, P. & ROBERTSON, G. P. 2006. The effect of increased n deposition on nitrous oxide, methane and carbon dioxide fluxes from unmanaged forest and grassland communities in Michigan. *Biogeochemistry,* 79**,** 315-337.

ANDERSON, L. J., DERNER, J. D., POLLEY, H. W., GORDON, W. S., EISSENSTAT, D. M. & JACKSON, R. B. 2010. Root responses along a subambient to elevated CO2 gradient in a C3â□“C4 grassland. *Global Change Biology,* 16**,** 454-468.

ANTONSEN, H. & OLSSON, P. A. 2005. Relative importance of burning, mowing and species translocation in the restoration of a former boreal hayfield: responses of plant diversity and the microbial community. *Journal of Applied Ecology,* 42**,** 337-347.

ARNETH, A., HARRISON, S. P., ZAEHLE, S., TSIGARIDIS, K., MENON, S., BARTLEIN, P. J., FEICHTER, J., KORHOLA, A., KULMALA, M., O'DONNELL, D., SCHURGERS, G., SORVARI, S. & VESALA, T. 2010. Terrestrial biogeochemical feedbacks in the climate system. *Nature Geoscience,* 3**,** 525.

ARONSON, E. L. & MCNULTY, S. G. 2009. Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. *Agricultural and Forest Meteorology,* 149**,** 1791-1799.

ASMAN, W. A. H., SUTTON, M. A. & SCHJØRRING, J. K. 1998. Ammonia: emission, atmospheric transport and deposition. *New Phytologist,* 139**,** 27-48.

BAGGS, E. M. & BLUM, H. 2004. CH4 oxidation and emissions of CH4 and N2O from Lolium perenne swards under elevated atmospheric CO2. *Soil Biology and Biochemistry,* 36**,** 713-723.

BAHN, M., KNAPP, M., GARAJOVA, Z., PFAHRINGER, N. & CERNUSCA, A. 2006. Root respiration in temperate mountain grasslands differing in land use. *Global Change Biology,* 12**,** 995-1006.

BAHN, M., RODEGHIERO, M., ANDERSON-DUNN, M., DORE, S., GIMENO, C., DRÖSLER, M., WILLIAMS, M., AMMANN, C., BERNINGER, F., FLECHARD, C., JONES, S., BALZAROLO, M., KUMAR, S., NEWESELY, C., PRIWITZER, T., RASCHI, A., SIEGWOLF, R., SUSILUOTO, S., TENHUNEN, J., WOHLFAHRT, G. & CERNUSCA, A. 2008. Soil Respiration in European Grasslands in Relation to Climate and Assimilate Supply. *Ecosystems (New York, N.Y.),* 11**,** 1352-67.

BAI, E., LI, S., XU, W., LI, W., DAI, W. & JIANG, P. 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytol,* 199**,** 431-40.

BAI, W., FANG, Y., ZHOU, M., XIE, T., LI, L. & ZHANG, W.-H. 2015. Heavily intensified grazing reduces root production in an Inner Mongolia temperate steppe. *Agriculture, Ecosystems & Environment,* 200**,** 143-150.

BAI, W., WAN, S., NIU, S., LIU, W., CHEN, Q., WANG, Q., ZHANG, W., HAN, X. & LI, L. 2010. Increased temperature and precipitation interact to affect root production, mortality, and turnover in a temperate steppe: implications for ecosystem C cycling. *Global Change Biology,* 16**,** 1306-1316.

BALL, B. C., SCOTT, A. & PARKER, J. P. 1999. Field N2O, CO2 and CH4 fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil and Tillage Research,* 53**,** 29-39.

BARDGETT, R. D. 2011. Plant-soil interactions in a changing world. *F1000 Biology Reports,* 3**,** 16.

BARDGETT, R. D., FREEMAN, C. & OSTLE, N. J. 2008. Microbial contributions to climate change through carbon cycle feedbacks. *ISME J,* 2**,** 805-814.

BARDGETT, R. D., MAWDSLEY, J. L., EDWARDS, S., HOBBS, P. J., RODWELL, J. S. & DAVIES, W. J. 1999. Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology,* 13**,** 650-660.

BARDGETT, R. D., WARDLE, D. A. & YEATES, G. W. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biology and Biochemistry,* 30**,** 1867-1878.

BATEMAN, E. J. & BAGGS, E. M. 2005. Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils,* 41**,** 379-388.

BAXENDALE, C., ORWIN, K. H., POLY, F., POMMIER, T. & BARDGETT, R. D. 2014. Are plant-soil feedback responses explained by plant traits? *New Phytol,* 204**,** 408-23.

BEHEYDT, D., BOECKX, P., SLEUTEL, S., LI, C. & VAN CLEEMPUT, O. 2007. Validation of DNDC for 22 long-term N2O field emission measurements. *Atmospheric Environment,* 41**,** 6196-6211.

BEIER, C., EMMETT, B. A., PEÑUELAS, J., SCHMIDT, I. K., TIETEMA, A., ESTIARTE, M., GUNDERSEN, P., LLORENS, L., RIIS-NIELSEN, T., SOWERBY, A. & GORISSEN, A. 2008. Carbon and nitrogen cycles in European ecosystems respond differently to global warming. *Science of The Total Environment,* 407**,** 692-697.

BEIS 2017. 2015 UK Greenhouse gas emissions. *In:* DEPARTMENT FOR BUSINESS, E. I. S. (ed.).

BENDER, S. F., CONEN, F. & VAN DER HEIJDEN, M. G. A. 2015. Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. *Soil Biology & Biochemistry,* 80**,** 283-292.

BENDER, S. F., PLANTENGA, F., NEFTEL, A., JOCHER, M., OBERHOLZER, H. R., KOHL, L., GILES, M., DANIELL, T. J. & VAN DER HEIJDEN, M. G. A. 2014. Symbiotic relationships between soil fungi and plants reduce N2O emissions from soil. *Isme Journal,* 8**,** 1336-1345.

BJORK, R. G., MAJDI, H., KLEMEDTSSON, L., LEWIS-JONSSON, L. & MOLAU, U. 2007. Long-term warming effects on root morphology, root mass distribution, and microbial activity in two dry tundra plant communities in northern Sweden. *New Phytol,* 176**,** 862-73.

BLANKE, V., BASSIN, S., VOLK, M. & FUHRER, J. 2012. Nitrogen deposition effects on subalpine grassland: The role of nutrient limitations and changes in mycorrhizal abundance. *Acta Oecologica,* 45**,** 57-65.

BLANKE, V., WAGNER, M., RENKER, C., LIPPERT, H., MICHULITZ, M., KUHN, A. J. & BUSCOT, F. 2011. Arbuscular mycorrhizas in phosphate-polluted soil: interrelations between root colonization and nitrogen. *Plant and Soil,* 343**,** 379-392.

BLANKINSHIP, J. C., BROWN, J. R., DIJKSTRA, P. & HUNGATE, B. A. 2010. Effects of interactive global changes on methane uptake in an annual grassland. *Journal of Geophysical Research-Biogeosciences,* 115.

BOLLER, B. C. & NÖSBERGER, J. 1987. Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low levels of15N-fertilization. *Plant and Soil,* 104**,** 219-226.

BOND-LAMBERTY, B. & THOMSON, A. 2010. Temperature-associated increases in the global soil respiration record. *Nature,* 464**,** 579.

BREMER, D. J., HAM, J. M., OWENSBY, C. E. & KNAPP, A. K. 1998. Responses of Soil Respiration to Clipping and Grazing in a Tallgrass Prairie. *Journal of Environmental Quality,* 27**,** 1539-1548.

BROWN, L., JARVIS, S. C. & HEADON, D. 2001. A farm-scale basis for predicting nitrous oxide emissions from dairy farms. *Nutrient Cycling in Agroecosystems,* 60**,** 149-158.

BROWN, L., SYED, B., JARVIS, S. C., SNEATH, R. W., PHILLIPS, V. R., GOULDING, K. W. T. & LI, C. 2002. Development and application of a mechanistic model to estimate emission of nitrous oxide from UK agriculture. *Atmospheric Environment,* 36**,** 917-928.

BRZOSTEK, E. R., BLAIR, J. M., DUKES, J. S., FREY, S. D., HOBBIE, S. E., MELILLO, J. M., MITCHELL, R. J., PENDALL, E., REICH, P. B., SHAVER, G. R., STEFANSKI, A., TJOELKER, M. G. & FINZI, A. C. 2012. The effect of experimental warming and precipitation change on proteolytic enzyme

activity: positive feedbacks to nitrogen availability are not universal. *Global Change Biology,* 18**,** 2617-2625.

BURCHILL, W., LANIGAN, G. J., LI, D., WILLIAMS, M. & HUMPHREYS, J. 2016. A system N balance for a pasture-based system of dairy production under moist maritime climatic conditions. *Agriculture, Ecosystems & Environment,* 220**,** 202-210.

BURTON, A. J., MELILLO, J. M. & FREY, S. D. 2008. Adjustment of Forest Ecosystem Root Respiration as Temperature Warms. *Journal of Integrative Plant Biology,* 50**,** 1467-1483.

BÜSCHER, M., ZAVALLONI, C., DE BOULOIS, H. D., VICCA, S., VAN DEN BERGE, J., DECLERCK, S., CEULEMANS, R., JANSSENS, I. A. & NIJS, I. 2012. Effects of arbuscular mycorrhizal fungi on grassland productivity are altered by future climate and below-ground resource availability. *Environmental and Experimental Botany,* 81**,** 62-71.

BUTTERBACH-BAHL, K., BAGGS, E. M., DANNENMANN, M., KIESE, R. & ZECHMEISTER-BOLTENSTERN, S. 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc Lond B Biol Sci,* 368**,** 20130122.

CAI, Z. C., SAWAMOTO, T., LI, C. S., KANG, G. D., BOONJAWAT, J., MOSIER, A., WASSMANN, R. & TSURUTA, H. 2003. Field validation of the DNDC model for greenhouse gas emissions in East Asian cropping systems. *Global Biogeochemical Cycles,* 17.

CANTAREL, A. A. M., BLOOR, J. M. G., POMMIER, T., GUILLAUMAUD, N., MOIROT, C., SOUSSANA, J.-F. & POLY, F. 2012. Four years of experimental climate change modifies the microbial drivers of N2O fluxes in an upland grassland ecosystem. *Global Change Biology,* 18**,** 2520-2531.

CAO, G., TANG, Y., MO, W., WANG, Y., LI, Y. & ZHAO, X. 2004. Grazing intensity alters soil respiration in an alpine meadow on the Tibetan plateau. *Soil Biology and Biochemistry,* 36**,** 237-243.

CARDENAS, L. M., THORMAN, R., ASHLEE, N., BUTLER, M., CHADWICK, D., CHAMBERS, B., CUTTLE, S., DONOVAN, N., KINGSTON, H. & LANE, S. 2010. Quantifying annual N2O emission fluxes from grazed grassland under a range of inorganic fertiliser nitrogen inputs. *Agriculture, Ecosystems & Environment,* 136**,** 218-226.

CAREY, P. D., WALLIS, S., CHAMBERLAIN, P. M., COOPER, A., EMMETT, B. A., MASKELL, L. C., MCCANN, T., MURPHY, J., NORTON, L. R., REYNOLDS, B., SCOTT, W. A., SIMPSON, I. C., SMART, S. M. & ULLYETT, J. M. 2008. Countryside Survey: UK Results from 2007. NERC/Centre for Ecology & Hydrology.

CARLSSON, G. & HUSS-DANELL, K. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil,* 253**,** 353-372.

CARLSSON, G., PALMBORG, C., JUMPPONEN, A., SCHERER-LORENZEN, M., HOGBERG, P. & HUSS-DANELL, K. 2009. N-2 fixation in three perennial Trifolium species in experimental grasslands of varied plant species richness and composition. *Plant Ecology,* 205**,** 87-104.

CARRILLO, Y., DIJKSTRA, F. A., LECAIN, D., MORGAN, J. A., BLUMENTHAL, D., WALDRON, S. & PENDALL, E. 2014. Disentangling root responses to climate change in a semiarid grassland. *Oecologia,* 175**,** 699-711.

CARTER, M. S. & AMBUS, P. 2006. Biologically Fixed N2 as a Source for N2O Production in a Grass–clover Mixture, Measured by 15N2. *Nutrient Cycling in Agroecosystems,* 74**,** 13-26.

CARTER, M. S., AMBUS, P., ALBERT, K. R., LARSEN, K. S., ANDERSSON, M., PRIEME, A., VAN DER LINDEN, L. & BEIER, C. 2011. Effects of elevated atmospheric CO2, prolonged summer drought and temperature increase on N2O and CH4 fluxes in a temperate heathland. *Soil Biology & Biochemistry,* 43**,** 1660-1670.

CASE, S. D. C., MCNAMARA, N. P., REAY, D. S. & WHITAKER, J. 2012. The effect of biochar addition on N2O and CO2 emissions from a sandy loam soil – The role of soil aeration. *Soil Biology and Biochemistry,* 51**,** 125-134.

CAVAGNARO, T. R., BARRIOS-MASIAS, F. H. & JACKSON, L. E. 2012. Arbuscular mycorrhizas and their role in plant growth, nitrogen interception and soil gas efflux in an organic production system. *Plant and Soil,* 353**,** 181-194.

CHADWICK, D. R., CARDENAS, L., MISSELBROOK, T. H., SMITH, K. A., REES, R. M., WATSON, C. J., MCGEOUGH, K. L., WILLIAMS, J. R., CLOY, J. M., THORMAN, R. E. & DHANOA, M. S. 2014. Optimizing chamber methods for measuring nitrous oxide emissions from plot-based agricultural experiments. *European Journal of Soil Science,* 65**,** 295-307.

CHEN, J., LUO, Y., XIA, J., SHI, Z., JIANG, L., NIU, S., ZHOU, X. & CAO, J. 2016a. Differential responses of ecosystem respiration components to experimental warming in a meadow grassland on the Tibetan Plateau. *Agricultural and Forest Meteorology,* 220**,** 21-29.

CHEN, S., LIN, S., REINSCH, T., LOGES, R., HASLER, M. & TAUBE, F. 2016b. Comparison of ingrowth core and sequential soil core methods for estimating belowground net primary production in grass-clover swards. *Grass and Forage Science,* 71**,** 515-528.

CHIRINDA, N., KRACHER, D., LÆGDSMAND, M., PORTER, J. R., OLESEN, J. E., PETERSEN, B. M., DOLTRA, J., KIESE, R. & BUTTERBACH-BAHL, K. 2011. Simulating soil N2O emissions and heterotrophic CO2 respiration in arable systems using FASSET and MoBiLE-DNDC. *Plant and Soil,* 343**,** 139-160.

CHIRINDA, N., OLESEN, J. E. & PORTER, J. R. 2012. Root carbon input in organic and inorganic fertilizer-based systems. *Plant and Soil,* 359**,** 321-333.

CHISTOSERDOVA, L., VORHOLT, J. A. & LIDSTROM, M. E. 2005. A genomic view of methane oxidation by aerobic bacteria and anaerobic archaea. *Genome Biol,* 6**,** 208.

CLEVELAND, C. C. & TOWNSEND, A. R. 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 10316-10321.

CRAINE, J. M., FROEHLE, J., TILMAN, G. D., WEDIN, D. A. & CHAPIN, F. S. 2001. The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos,* 93**,** 274-285.

CROWTHER, T. W. & BRADFORD, M. A. 2013. Thermal acclimation in widespread heterotrophic soil microbes. *Ecology Letters,* 16**,** 469-477.

CURIEL YUSTE, J., JANSSENS, I. A., CARRARA, A. & CEULEMANS, R. 2004. Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity. *Global Change Biology,* 10**,** 161-169.

CURRY, C. L. 2007. Modeling the soil consumption of atmospheric methane at the global scale. *Global Biogeochemical Cycles,* 21.

DAVIDSON, E. A., ISHIDA, F. Y. & NEPSTAD, D. C. 2004. Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology,* 10**,** 718-730.

DAVIDSON, E. A. & JANSSENS, I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature,* 440**,** 165-173.

DAVIDSON, E. A., SAVAGE, K., BOLSTAD, P., CLARK, D. A., CURTIS, P. S., ELLSWORTH, D. S., HANSON, P. J., LAW, B. E., LUO, Y., PREGITZER, K. S., RANDOLPH, J. C. & ZAK, D. 2002. Belowground carbon allocation in forests estimated from litterfall and IRGA-based soil respiration measurements. *Agricultural and Forest Meteorology,* 113**,** 39-51.

DE DEYN, G. B., CORNELISSEN, J. H. C. & BARDGETT, R. D. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters,* 11**,** 516-531.

DE DEYN, G. B., QUIRK, H., YI, Z., OAKLEY, S., OSTLE, N. J. & BARDGETT, R. D. 2009. Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. *Journal of Ecology,* 97**,** 864-875.

DE DEYN, G. B., SHIEL, R. S., OSTLE, N. J., MCNAMARA, N. P., OAKLEY, S., YOUNG, I., FREEMAN, C., FENNER, N., QUIRK, H. & BARDGETT, R. D. 2011. Additional carbon sequestration benefits of grassland diversity restoration. *Journal of Applied Ecology,* 48**,** 600-608.

DE VRIES, F. T., MANNING, P., TALLOWIN, J. R. B., MORTIMER, S. R., PILGRIM, E. S., HARRISON, K. A., HOBBS, P. J., QUIRK, H., SHIPLEY, B., CORNELISSEN, J. H. C., KATTGE, J. & BARDGETT, R. D. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. *Ecology Letters,* 15**,** 1230-1239.

DE VRIES, W., KROS, J., KUIKMAN, P. J., VELTHOF, G. L., VOOGD, J. C. H., WIEGGERS, H. J. J., BUTTERBACH-BAHL, K., DENIER VAN DER GON, H. A. C. & VAN AMSTEL, A. R. 2005. Use of measurements and models to improve the national IPCC based assessments of soil emissions of nitrous oxide. *Environmental Sciences,* 2**,** 217-233.

DENTON, C. S., BARDGETT, R. D., COOK, R. & HOBBS, P. J. 1998. Low amounts of root herbivory positively influence the rhizosphere microbial community in a temperate grassland soil. *Soil Biology and Biochemistry,* 31**,** 155-165.

DI, H. J. & CAMERON, K. C. 2002. Nitrate leaching and pasture production from different nitrogen sources on a shallow stoney soil under flood-irrigated dairy pasture. *Australian Journal of Soil Research,* 40**,** 317-334.

DIELEMAN, W. I. J., VICCA, S., DIJKSTRA, F. A., HAGEDORN, F., HOVENDEN, M. J., LARSEN, K. S., MORGAN, J. A., VOLDER, A., BEIER, C., DUKES, J. S., KING, J., LEUZINGER, S., LINDER, S., LUO, Y., OREN, R., DE ANGELIS, P., TINGEY, D., HOOSBEEK, M. R. & JANSSENS, I. A. 2012. Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO2 and temperature. *Global Change Biology,* 18**,** 2681-2693.

DIJKSTRA, F. A., CARRILLO, Y., PENDALL, E. & MORGAN, J. A. 2013a. Rhizosphere priming: a nutrient perspective. *Front Microbiol,* 4**,** 216.

DIJKSTRA, F. A., MORGAN, J. A., FOLLETT, R. F. & LECAIN, D. R. 2013b. Climate change reduces the net sink of CH4 and N2O in a semiarid grassland. *Glob Chang Biol,* 19**,** 1816-26.

DIJKSTRA, F. A., MORGAN, J. A., VON FISCHER, J. C. & FOLLETT, R. F. 2011. Elevated CO2 and warming effects on CH4 uptake in a semiarid grassland below optimum soil moisture. *Journal of Geophysical Research: Biogeosciences,* 116**,** n/a-n/a.

DIJKSTRA, F. A., PRIOR, S. A., RUNION, G. B., TORBERT, H. A., TIAN, H., LU, C. & VENTEREA, R. T. 2012. Effects of elevated carbon dioxide and increased temperature on methane and nitrous oxide fluxes: evidence from field experiments. *Frontiers in Ecology and the Environment,* 10**,** 520-527.

DOBBIE, K. E. & SMITH, K. A. 2001. The effects of temperature, water-filled pore space and land use on N2O emissions from an imperfectly drained gleysol. *European Journal of Soil Science,* 52**,** 667-673.

DONNELLY, D. P., BODDY, L. & LEAKE, J. R. 2004. Development, persistence and regeneration of foraging ectomycorrhizal mycelial systems in soil microcosms. *Mycorrhiza,* 14**,** 37-45.

DRIGO, B., PIJL, A. S., DUYTS, H., KIELAK, A. M., GAMPER, H. A., HOUTEKAMER, M. J., BOSCHKER, H. T. S., BODELIER, P. L. E., WHITELEY, A. S., VAN VEEN, J. A. & KOWALCHUK, G. A. 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO(2). *Proceedings of the National Academy of Sciences of the United States of America,* 107**,** 10938-10942.

DRINKWATER, L. E., WAGONER, P. & SARRANTONIO, M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature,* 396**,** 262-265.

DU, F., SUI, Y. Y., XU, X. X., ZHANG, X. C., HAO, W. F., SHAO, M. G., HU, L. J. & SHAN, L. 2013. Morhpological Responses of Six Plant Species to Patchy Habitat with Different Nitrogen Concentrations Using in-Growth Core Method. *Polish Journal of Ecology,* 61**,** 257-269.

DUNFIELD, P. & KNOWLES, R. 1995. Kinetics of Inhibition of Methane Oxidation by Nitrate, Nitrite, and Ammonium in a Humisol. *Applied and Environmental Microbiology,* 61**,** 3129-3135.

DUXBURY, J. M., HARPER, L. A. & MOSIER, A. R. 1993. Contributions of Agroecosystems to Global Climate Change. *Agricultural Ecosystem Effects on Trace Gases and Global Climate Change,* 55**,** 1-18.

EISSENSTAT, D. M. 1992. Costs and Benefits of Constructing Roots of Small Diameter. *Journal of Plant Nutrition,* 15**,** 763-782.

EISSENSTAT, D. M., WELLS, C. E., YANAI, R. D. & WHITBECK, J. L. 2000. Building roots in a changing environment: implications for root longevity. *New Phytologist,* 147**,** 33-42.

EMMETT, B. A., BEIER, C., ESTIARTE, M., TIETEMA, A., KRISTENSEN, H. L., WILLIAMS, D., PENUELAS, J., SCHMIDT, I. & SOWERBY, A. 2004. The response of soil processes to climate change: Results from manipulation studies of shrublands across an environmental gradient. *Ecosystems,* 7**,** 625-637.

ERB, K. H., LAUK, C., KASTNER, T., MAYER, A., THEURL, M. C. & HABERL, H. 2016. Exploring the biophysical option space for feeding the world without deforestation. *Nature Communications,* 7.

FANG, C. & MONCRIEFF, J. B. 2001. The dependence of soil CO2 efflux on temperature. *Soil Biology & Biochemistry,* 33**,** 155-165.

FAO. 2015. *Food and Agriculture Organisation of the United Nations* [Online]. Available: http://www.fao.org/home/en/ [Accessed 16 October 2017].
FAREWELL, T. S., TRUCKELL, I. G., KEAY, C. A. & HALLETT, S. H. 2011. Use and applications of the Soilscapes datasets, Cranfield University.
FETZEL, T., HAVLIK, P., HERRERO, M. & ERB, K.-H. 2017. Seasonality constraints to livestock grazing intensity. *Global Change Biology,* 23**,** 1636-1647.
FIRESTONE, M. K. & DAVIDSON, E. A. 1989. Microbiological Basis of No and N2o Production and Consumption in Soil. *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere,* 47**,** 7-21.
FITTON, N., DATTA, A., CLOY, J. M., REES, R. M., TOPP, C. F. E., BELL, M. J., CARDENAS, L. M., WILLIAMS, J., SMITH, K., THORMAN, R., WATSON, C. J., MCGEOUGH, K. L., KUHNERT, M., HASTINGS, A., ANTHONY, S., CHADWICK, D. & SMITH, P. 2017. Modelling spatial and inter-annual variations of nitrous oxide emissions from UK cropland and grasslands using DailyDayCent. *Agriculture, Ecosystems & Environment,* 250**,** 1-11.
FONTAINE, S., BARDOUX, G., ABBADIE, L. & MARIOTTI, A. 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters,* 7**,** 314-320.
FORNARA, D. A. & TILMAN, D. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology,* 96**,** 314-322.
FORNARA, D. A. & TILMAN, D. 2012. Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. *Ecology,* 93**,** 2030-2036.
FOSTER, B. L., KINDSCHER, K., HOUSEMAN, G. R. & MURPHY, C. A. 2009. Effects of hay management and native species sowing on grassland community structure, biomass, and restoration. *Ecol Appl,* 19**,** 1884-96.
FU, J., GASCHE, R., WANG, N., LU, H., BUTTERBACH-BAHL, K. & KIESE, R. 2017. Impacts of climate and management on water balance and nitrogen leaching from montane grassland soils of S-Germany. *Environ Pollut,* 229**,** 119-131.
GALLOWAY, J. N., WINIWARTER, W., LEIP, A., LEACH, A. M., BLEEKER, A. & ERISMAN, J. W. 2014. Nitrogen footprints: past, present and future. *Environmental Research Letters,* 9.
GAO, Y. H., LUO, P., WU, N., CHEN, H. & WANG, G. X. 2008a. Impacts of Grazing Intensity on Nitrogen Pools and Nitrogen Cycle in an Alpine Meadow on the Eastern Tibetan Plateau. *Applied Ecology and Environmental Research,* 6**,** 69-79.
GAO, Y. Z., CHEN, Q., LIN, S., GIESE, M. & BRUECK, H. 2011. Resource manipulation effects on net primary production, biomass allocation and rain-use efficiency of two semiarid grassland sites in Inner Mongolia, China. *Oecologia,* 165**,** 855-64.
GAO, Y. Z., GIESE, M., LIN, S., SATTELMACHER, B., ZHAO, Y. & BRUECK, H. 2008b. Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. *Plant and Soil,* 307**,** 41-50.
GARNETT, T. 2009. Livestock-related greenhouse gas emissions: impacts and options for policy makers. *Environmental Science & Policy,* 12**,** 491-503.
GARNETT, T., APPLEBY, M. C., BALMFORD, A., BATEMAN, I. J., BENTON, T. G., BLOOMER, P., BURLINGAME, B., DAWKINS, M., DOLAN, L., FRASER, D., HERRERO, M., HOFFMANN, I., SMITH, P., THORNTON, P. K., TOULMIN, C., VERMEULEN, S. J. & GODFRAY, H. C. 2013.

Agriculture. Sustainable intensification in agriculture: premises and policies. *Science,* 341**,** 33-4.
GAVITO, M. E. & OLSSON, P. A. 2003. Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol,* 45**,** 181-7.
GIARDINA, C. P., BINKLEY, D., RYAN, M. G., FOWNES, J. H. & SENOCK, R. S. 2004. Belowground carbon cycling in a humid tropical forest decreases with fertilization. *Oecologia,* 139**,** 545-550.
GIARDINA, C. P., RYAN, M. G., BINKLEY, D. & FOWNES, J. H. 2003. Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology,* 9**,** 1438-1450.
GIBSON, D. J. 2008. *Grasses and Grassland Ecology,* Oxford, Oxford University Press.
GIESE, M., BRUECK, H., GAO, Y. Z., LIN, S., STEFFENS, M., KÖGEL-KNABNER, I., GLINDEMANN, T., SUSENBETH, A., TAUBE, F., BUTTERBACH-BAHL, K., ZHENG, X. H., HOFFMANN, C., BAI, Y. F. & HAN, X. G. 2013. N balance and cycling of Inner Mongolia typical steppe: a comprehensive case study of grazing effects. *Ecological Monographs,* 83**,** 195-219.
GILAD, O. 2008. Competition and Competition Models A2 - Jørgensen, Sven Erik. *In:* FATH, B. D. (ed.) *Encyclopedia of Ecology.* Oxford: Academic Press.
GILHESPY, S. L., ANTHONY, S., CARDENAS, L., CHADWICK, D., DEL PRADO, A., LI, C. S., MISSELBROOK, T., REES, R. M., SALAS, W., SANZ-COBENA, A., SMITH, P., TILSTON, E. L., TOPP, C. F. E., VETTER, S. & YELURIPATI, J. B. 2014. First 20 years of DNDC (DeNitrification DeComposition): Model evolution. *Ecological Modelling,* 292**,** 51-62.
GILL, R. A. 2014. The influence of 3-years of warming and N-deposition on ecosystem dynamics is small compared to past land use in subalpine meadows. *Plant and Soil,* 374**,** 197-210.
GILLSON, L. & HOFFMAN, M. T. 2007. Rangeland Ecology in a Changing World. *Science,* 315**,** 53-54.
GILTRAP, D. L., LI, C. & SAGGAR, S. 2010. DNDC: A process-based model of greenhouse gas fluxes from agricultural soils. *Agriculture, Ecosystems & Environment,* 136**,** 292-300.
GOUGOULIAS, C., CLARK, J. M. & SHAW, L. J. 2014. The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *J Sci Food Agric,* 94**,** 2362-71.
GOVINDARAJULU, M., PFEFFER, P. E., JIN, H., ABUBAKER, J., DOUDS, D. D., ALLEN, J. W., BUCKING, H., LAMMERS, P. J. & SHACHAR-HILL, Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature,* 435**,** 819-823.
GRAHAM, S. L., HUNT, J. E., MILLARD, P., MCSEVENY, T., TYLIANAKIS, J. M. & WHITEHEAD, D. 2014. Effects of soil warming and nitrogen addition on soil respiration in a New Zealand tussock grassland. *PLoS One,* 9**,** e91204.
GRAY, S. B. & BRADY, S. M. 2016. Plant developmental responses to climate change. *Developmental Biology,* 419**,** 64-77.
GRIGULIS, K., LAVOREL, S., KRAINER, U., LEGAY, N., BAXENDALE, C., DUMONT, M., KASTL, E., ARNOLDI, C., BARDGETT, R. D., POLY, F., POMMIER, T., SCHLOTER, M., TAPPEINER, U., BAHN, M., CLÉMENT, J.-C. & HUTCHINGS, M. 2013. Relative contributions of plant traits and soil

microbial properties to mountain grassland ecosystem services. *Journal of Ecology,* 101**,** 47-57.
GROSSO, S. J. D., PARTON, W. J., MOSIER, A. R., OJIMA, D. S., POTTER, C. S., BORKEN, W., BRUMME, R., BUTTERBACH-BAHL, K., CRILL, P. M., DOBBIE, K. & SMITH, K. A. 2000. General CH4 oxidation model and comparisons of CH4 Oxidation in natural and managed systems. *Global Biogeochemical Cycles,* 14**,** 999-1019.
GUITIAN, R. & BARDGETT, R. D. 2000. Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil,* 220**,** 271.
GULZARI, S. O., ABY, B. A., PERSSON, T., HOGLIND, M. & MITTENZWEI, K. 2017. Combining models to estimate the impacts of future climate scenarios on feed supply, greenhouse gas emissions and economic performance on dairy farms in Norway. *Agricultural Systems,* 157**,** 157-169.
GUSEWELL, S., JEWELL, P. L. & EDWARDS, P. J. 2005. Effects of heterogeneous habitat use by cattle on nutrient availability and litter decomposition in soils of an Alpine pasture. *Plant and Soil,* 268**,** 135-149.
HAMILTON, E. W. & FRANK, D. A. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology,* 82**,** 2397-2402.
HAN, Q., LUO, G., LI, C. & XU, W. 2014. Modeling the grazing effect on dry grassland carbon cycling with Biome-BGC model. *Ecological Complexity,* 17**,** 149-157.
HAN, Y., ZHANG, Z., WANG, C. H., JIANG, F. H. & XIA, J. Y. 2012. Effects of mowing and nitrogen addition on soil respiration in three patches in an oldfield grassland in Inner Mongolia. *Journal of Plant Ecology,* 5**,** 219-228.
HANSON ET AL. 2000. Separating root and soil microbial contributions to soil respiration: A review ofmethods and observations

HARTMANN, A. A., BUCHMANN, N. & NIKLAUS, P. A. 2011. A study of soil methane sink regulation in two grasslands exposed to drought and N fertilization. *Plant and Soil,* 342**,** 265-275.
HAYNES, R. J. 1980. Competitive Aspects of the Grass-Legume Association. *Advances in Agronomy,* 33**,** 227-261.
HEINEMEYER, A. & FITTER, A. H. 2004. Impact of temperature on the arbuscular mycorrhizal (AM) symbiosis: growth responses of the host plant and its AM fungal partner. *J Exp Bot,* 55**,** 525-34.
HEINEMEYER, A., HARTLEY, I. P., EVANS, S. P., DE LA FUENTE, J. A. C. & INESON, P. 2007. *Forest soil CO2 flux: uncovering the contribution and environmental responses of ectomycorrhizas.* 13.
HEINEMEYER, A., INESON, P., OSTLE, N. & FITTER, A. H. 2006. Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytol,* 171**,** 159-70.
HEINEMEYER, A., TORTORELLA, D., PETROVIČOVÁ, B. & GELSOMINO, A. 2012. Partitioning of soil CO2 flux components in a temperate grassland ecosystem. *European Journal of Soil Science,* 63**,** 249-260.
HEYDE, M. V. D., BENNETT, J. A., PITHER, J. & HART, M. 2017. Longterm effects of grazing on arbuscular mycorrhizal fungi. *Agriculture, Ecosystems & Environment,* 243**,** 27-33.

HILLSTROM, M., MEEHAN, T. D., KELLY, K. & LINDROTH, R. L. 2010. Soil carbon and nitrogen mineralization following deposition of insect frass and greenfall from forests under elevated CO2 and O3. *Plant and Soil,* 336**,** 75-85.

HODGE, A., CAMPBELL, C. D. & FITTER, A. H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature,* 413**,** 297-299.

HODGE, A., HELGASON, T. & FITTER, A. H. 2010. Nutritional ecology of arbuscular mycorrhizal fungi. *Fungal Ecology,* 3**,** 267-273.

HODGE, A., ROBINSON, D. & FITTER, A. 2000. Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science,* 5**,** 304-308.

HÖGBERG, M. N., MYROLD, D. D., GIESLER, R. & HÖGBERG, P. 2006. Contrasting patterns of soil N-cycling in model ecosystems of Fennoscandian boreal forests. *Oecologia,* 147**,** 96-107.

HØGH-JENSEN, H. & SCHJOERRING, J. K. 1997. Interactions between white clover and ryegrass under contrasting nitrogen availability: N2 fixation, N fertilizer recovery, N transfer and water use efficiency. *Plant and Soil,* 197**,** 187-199.

HOLLAND, E. A., ROBERTSON, G. P., GREENBERG, J., GROFFMAN, P., BOONE, R. & GOSZ, J. 1999. Soil CO2, N2O, and CH4 Exchange. *In:* SOLLINS, P. (ed.) *Standard Soil Methods for Long-Term Ecological Research.* New York: Oxford University Press.

HOLZ, M., AURANGOJEB, M., KASIMIR, A., BOECKX, P., KUZYAKOV, Y., KLEMEDTSSON, L. & RUTTING, T. 2016. Gross Nitrogen Dynamics in the Mycorrhizosphere of an Organic Forest Soil. *Ecosystems,* 19**,** 284-295.

HOPKINS, F. M., TORN, M. S. & TRUMBORE, S. E. 2012. Warming accelerates decomposition of decades-old carbon in forest soils. *Proceedings of the National Academy of Sciences of the United States of America,* 109**,** E1753-E1761.

HORZ, H. P., RICH, V., AVRAHAMI, S. & BOHANNAN, B. J. 2005. Methane-oxidizing bacteria in a California upland grassland soil: diversity and response to simulated global change. *Appl Environ Microbiol,* 71**,** 2642-52.

HOYLE, F. C. & MURPHY, D. V. 2011. Influence of organic residues and soil incorporation on temporal measures of microbial biomass and plant available nitrogen. *Plant and Soil,* 347**,** 53-64.

HSIEH, C.-I., LEAHY, P., KIELY, G. & LI, C. 2005. The Effect of Future Climate Perturbations on N2O Emissions from a Fertilized Humid Grassland. *Nutrient Cycling in Agroecosystems,* 73**,** 15-23.

HUANG, Y., ZOU, J., ZHENG, X., WANG, Y. & XU, X. 2004. Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. *Soil Biology and Biochemistry,* 36**,** 973-981.

HÜTSCH, B. W. 1998. Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. *Biology and Fertility of Soils,* 28**,** 27-35.

IMER, D., MERBOLD, L., EUGSTER, W. & BUCHMANN, N. 2013. Temporal and spatial variations of soil CO₂, CH₄ and N₂O fluxes at three differently managed grasslands. *Biogeosciences,* 10**,** 5931-5945.

IPCC 2000. Special Report on Emission Scenarios. *In:* NAKICENOVIC, N. & SWART, S. E. (eds.) *A Special Report of Working Group III of the Intergovernmental Panel on Climate Change.* Cambridge, UK: Cambridge University Press, Cambridge, UK.

IPCC 2007. Summary for policymakers. . *In:* SOLOMON, S., QIN, D., MANNING, M., CHEN, Z., MARQUIS, M., AVERYT, K. B., TIGNOR, M. & MILLER, H. L. (eds.) *Climate change 2007: The physical science basis. In Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press: Cambridge, UK.

IPCC 2013. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* Cambridge, United Kingdom and New York, NY, USA, Cambridge University Press.

IPCC 2014. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,* Geneva, Switzerland, IPCC.

JABRO, J. D., SAINJU, U., STEVENS, W. B. & EVANS, R. G. 2008. Carbon dioxide flux as affected by tillage and irrigation in soil converted from perennial forages to annual crops. *Journal of Environmental Management,* 88**,** 1478-1484.

JACKSON, L. E., SCHIMEL, J. P. & FIRESTONE, M. K. 1989. Short-Term Partitioning of Ammonium and Nitrate between Plants and Microbes in an Annual Grassland. *Soil Biology & Biochemistry,* 21**,** 409-415.

JANG, I., LEE, S., ZOH, K. D. & KANG, H. 2011. Methane concentrations and methanotrophic community structure influence the response of soil methane oxidation to nitrogen content in a temperate forest. *Soil Biology & Biochemistry,* 43**,** 620-627.

JIANG, C., YU, G., FANG, H., CAO, G. & LI, Y. 2010. Short-term effect of increasing nitrogen deposition on CO2, CH4 and N2O fluxes in an alpine meadow on the Qinghai-Tibetan Plateau, China. *Atmospheric Environment,* 44**,** 2920-2926.

JIN, H., PFEFFER, P. E., DOUDS, D. D., PIOTROWSKI, E., LAMMERS, P. J. & SHACHAR-HILL, Y. 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytologist,* 168**,** 687-696.

JOHNSON, D., LEAKE, J. R., OSTLE, N., INESON, P. & READ, D. J. 2002a. In situ (CO2)-C-13 pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytologist,* 153**,** 327-334.

JOHNSON, D., LEAKE, J. R. & READ, D. J. 2001. Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. *New Phytologist,* 152**,** 555-562.

JOHNSON, D., LEAKE, J. R. & READ, D. J. 2002b. Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of 14C. *Soil Biology and Biochemistry,* 34**,** 1521-1524.

JOHNSON, D., LEAKE, J. R., READ, D. J. & GADD, G. M. 2006. Role of arbuscular mycorrhizal fungi in carbon and nutrient cycling in grassland. 129-150.

JOHNSON, N. C., TILMAN, D. & WEDIN, D. 1992. Plant and Soil Controls on Mycorrhizal Fungal Communities. *Ecology,* 73**,** 2034-2042.

JONES, D. L., HEALEY, J. R., WILLETT, V. B., FARRAR, J. F. & HODGE, A. 2005a. Dissolved organic nitrogen uptake by plants—an important N uptake pathway? *Soil Biology and Biochemistry,* 37**,** 413-423.

JONES, D. L., HODGE, A. & KUZYAKOV, Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist,* 163**,** 459-480.

JONES, D. L., NGUYEN, C. & FINLAY, R. D. 2009. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant and Soil,* 321**,** 5-33.

JONES, S. K., REES, R. M., SKIBA, U. M. & BALL, B. C. 2005b. Greenhouse gas emissions from a managed grassland. *Global and Planetary Change,* 47**,** 201-211.

JONES, S. K., REES, R. M., SKIBA, U. M. & BALL, B. C. 2007. Influence of organic and mineral N fertiliser on N2O fluxes from a temperate grassland. *Agriculture, Ecosystems & Environment,* 121**,** 74-83.

KAHKONEN, M. A., WITTMANN, C., ILVESNIEMI, H., WESTMAN, C. J. & SALKINOJA-SALONEN, M. S. 2002. Mineralization of detritus and oxidation of methane in acid boreal coniferous forest soils: seasonal and vertical distribution and effects of clear-cut. *Soil Biology & Biochemistry,* 34**,** 1191-1200.

KALLENBACH, C. M., FREY, S. D. & GRANDY, A. S. 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications,* 7**,** 13630.

KANG, X. M., HAO, Y. B., CUI, X. Y., CHEN, H., LI, C. S., RUI, Y. C., TIAN, J. Q., KARDOL, P., ZHONG, L., WANG, J. Z. & WANG, Y. F. 2013. Effects of grazing on CO2 balance in a semiarid steppe: field observations and modeling. *Journal of Soils and Sediments,* 13**,** 1012-1023.

KASCHUK, G., LEFFELAAR, P. A., GILLER, K. E., ALBERTON, O., HUNGRIA, M. & KUYPER, T. W. 2010. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. *Soil Biology and Biochemistry,* 42**,** 125-127.

KAYE, J. P. & HART, S. C. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution,* 12**,** 139-143.

KESIK, M., BRÜGGEMANN, N., FORKEL, R., KIESE, R., KNOCHE, R., LI, C., SEUFERT, G., SIMPSON, D. & BUTTERBACH-BAHL, K. 2006. Future scenarios of N2O and NO emissions from European forest soils. *Journal of Geophysical Research: Biogeosciences,* 111**,** n/a-n/a.

KIDD, J., MANNING, P., SIMKIN, J., PEACOCK, S. & STOCKDALE, E. 2017. Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. *PLOS ONE,* 12**,** e0174632.

KIM, D.-G., HERNANDEZ-RAMIREZ, G. & GILTRAP, D. 2013. Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis. *Agriculture, Ecosystems & Environment,* 168**,** 53-65.

KIRSCHBAUM, M. U. F. 2004. Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology,* 10**,** 1870-1877.

KIRWAN, L., LUESCHER, A., SEBASTIA, M. T., FINN, J. A., COLLINS, R. P., PORQUEDDU, C., HELGADOTTIR, A., BAADSHAUG, O. H., BROPHY, C., CORAN, C., DALMANNSDOTTIR, S., DELGADO, I., ELGERSMA, A., FOTHERGILL, M., FRANKOW-LINDBERG, B. E., GOLINSKI, P., GRIEU, P., GUSTAVSSON, A. M., HOGLIND, M., HUGUENIN-ELIE, O., ILIADIS, C., JORGENSEN, M., KADZIULIENE, Z., KARYOTIS, T., LUNNAN, T., MALENGIER, M., MALTONI, S., MEYER, V., NYFELER, D., NYKANEN-KURKI, P., PARENTE, J., SMIT, H. J., THUMM, U. & CONNOLLY, J. 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology,* 95**,** 530-539.

KOOL, D. M., DOLFING, J., WRAGE, N. & VAN GROENIGEN, J. W. 2011. Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology & Biochemistry,* 43**,** 174-178.

KRÖBEL, R., SUN, Q., INGWERSEN, J., CHEN, X., ZHANG, F., MÜLLER, T. & RÖMHELD, V. 2010. Modelling water dynamics with DNDC and DAISY in a soil of the North China Plain: A comparative study. *Environmental Modelling & Software,* 25**,** 583-601.

KUZYAKOV, Y. 2002. Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* 165**,** 382-396.

KUZYAKOV, Y., BIRYUKOVA, O. V., KUZNETZOVA, T. V., MOLTER, K., KANDELER, E. & STAHR, K. 2002. Carbon partitioning in plant and soil, carbon dioxide fluxes and enzyme activities as affected by cutting ryegrass. *Biology and Fertility of Soils,* 35**,** 348-358.

KUZYAKOV, Y. & BOL, R. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biology and Biochemistry,* 38**,** 747-758.

LAIDLAW, A. S., CHRISTIE, P. & LEE, H. W. 1996. Effect of white clover cultivar on apparent transfer of nitrogen from clover to grass and estimation of relative turnover rates of nitrogen in roots. *Plant and Soil,* 179**,** 243-253.

LAL, R. 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science,* 304**,** 1623-1627.

LAVOREL, S. & GARNIER, E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology,* 16**,** 545-556.

LAVOREL, S., STORKEY, J., BARDGETT, R. D., DE BELLO, F., BERG, M. P., LE ROUX, X., MORETTI, M., MULDER, C., PAKEMAN, R. J., DÍAZ, S. & HARRINGTON, R. 2013. A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. *Journal of Vegetation Science,* 24**,** 942-948.

LAZCANO, C., BARRIOS-MASIAS, F. H. & JACKSON, L. E. 2014. Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. *Soil Biology and Biochemistry,* 74**,** 184-192.

LE MER, J. & ROGER, P. 2001. Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology,* 37**,** 25-50.

LEAKE, J., JOHNSON, D., DONNELLY, D., MUCKLE, G., BODDY, L. & READ, D. 2004. Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Canadian Journal of Botany,* 82**,** 1016-1045.

LECAIN, D. R., MORGAN, J. A., SCHUMAN, G. E., REEDER, J. D. & HART, R. H. 2002. Carbon exchange and species composition of grazed pastures and exclosures in the shortgrass steppe of Colorado. *Agriculture, Ecosystems & Environment,* 93**,** 421-435.

LEDGARD, S., SCHILS, R., ERIKSEN, J. & LUO, J. 2009. Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research,* 48**,** 209-226.

LEDGARD, S. F. 2001. Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil,* 228**,** 43-59.

LEDGARD, S. F., SPROSEN, M. S., PENNO, J. W. & RAJENDRAM, G. S. 2001. Nitrogen fixation by white clover in pastures grazed by dairy cows: Temporal variation and effects of nitrogen fertilization. *Plant and Soil,* 229**,** 177-187.

LEDGARD, S. F. & STEELE, K. W. 1992. Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil,* 141**,** 137-153.

LEE, M., MANNING, P., RIST, J., POWER, S. A. & MARSH, C. 2010. A global comparison of grassland biomass responses to CO2 and nitrogen enrichment. *Philos Trans R Soc Lond B Biol Sci,* 365**,** 2047-56.

LERICHE, H., LEROUX, X., GIGNOUX, J., TUZET, A., FRITZ, H., ABBADIE, L. & LOREAU, M. 2001. Which functional processes control the short-term effect of grazing on net primary production in grasslands? *Oecologia,* 129**,** 114-124.

LEVY, P. E., MOBBS, D. C., JONES, S. K., MILNE, R., CAMPBELL, C. & SUTTON, M. A. 2007. Simulation of fluxes of greenhouse gases from European grasslands using the DNDC model. *Agriculture, Ecosystems & Environment,* 121**,** 186-192.

LI, C. 2013. *User's guide for the DNDC model (v. 9.5)* [Online]. Available: http://www.dndc.sr.unh.edu/ [Accessed 24/11/2017].

LI, C., FROLKING, S. & FROLKING, T. A. 1992. A model of nitrous oxide evolution from soil driven by rainfall events: 1. Model structure and sensitivity. *Journal of Geophysical Research: Atmospheres,* 97**,** 9759-9776.

LI, C., FROLKING, S. & HARRISS, R. 1994. Modeling carbon biogeochemistry in agricultural soils. *Global Biogeochemical Cycles,* 8**,** 237-254.

LI, C., ZHUANG, Y., CAO, M., CRILL, P., DAI, Z., FROLKING, S., MOORE, B., SALAS, W., SONG, W. & WANG, X. 2001. Comparing a process-based agro-ecosystem model to the IPCC methodology for developing a national inventory of N2O emissions from arable lands in China. *Nutrient Cycling in Agroecosystems,* 60**,** 159-175.

LI, C. S. 2000. Modeling Trace Gas Emissions from Agricultural Ecosystems. *Nutrient Cycling in Agroecosystems,* 58**,** 259-276.

LI, C. S., ABER, J., STANGE, F., BUTTERBACH-BAHL, K. & PAPEN, H. 2000. A process-oriented model of N2O and NO emissions from forest soils: 1. Model development. *Journal of Geophysical Research-Atmospheres,* 105**,** 4369-4384.

LI, H., WANG, L. G. & QIU, J. J. 2007. [Estimation of CO2 and N2O emissions from farmland soils in Hebei Province in Huang-Huai-Hai Plain]. *Ying Yong Sheng Tai Xue Bao,* 18**,** 1994-2000.

LI, J., ZHANG, Q., LI, Y., LIU, Y., XU, J. & DI, H. 2017a. Effects of long-term mowing on the fractions and chemical composition of soil organic matter in a semiarid grassland. *Biogeosciences,* 14**,** 2685-2696.

LI, Q., SONG, Y., LI, G., YU, P., WANG, P. & ZHOU, D. 2015. Grass-legume mixtures impact soil N, species recruitment, and productivity in temperate steppe grassland. *Plant and Soil,* 394**,** 271-285.

LI, Q., YU, P. J., LI, G. D. & ZHOU, D. W. 2016. Grass-legume ratio can change soil carbon and nitrogen storage in a temperate steppe grassland. *Soil & Tillage Research,* 157**,** 23-31.

LI, R. H., LI, X. B., LI, G. Q. & WEN, W. Y. 2014. Simulation of soil nitrogen storage of the typical steppe with the DNDC model: A case study in Inner Mongolia, China. *Ecological Indicators,* 41**,** 155-164.

LI, X. L., GEORGE, E. & MARSCHNER, H. 1991. Phosphorus Depletion and Ph Decrease at the Root Soil and Hyphae Soil Interfaces of Va Mycorrhizal White Clover Fertilized with Ammonium. *New Phytologist,* 119**,** 397-404.

LI, Z., YANG, J. Y., DRURY, C. F., YANG, X. M., REYNOLDS, W. D., LI, X. & HU, C. 2017b. Evaluation of the DNDC model for simulating soil temperature, moisture and respiration from monoculture and rotational corn, soybean and winter wheat in Canada. *Ecological Modelling,* 360**,** 230-243.

LIANG, C. & BALSER, T. C. 2011. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. *Nat Rev Micro,* 9**,** 75-75.

LILLESKOV, E. A., HOBBIE, E. A. & HORTON, T. R. 2011. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology,* 4**,** 174-183.

LIU, C., HOLST, J., BRÜGGEMANN, N., BUTTERBACH-BAHL, K., YAO, Z., YUE, J., HAN, S., HAN, X., KRÜMMELBEIN, J., HORN, R. & ZHENG, X. 2007. Winter-grazing reduces methane uptake by soils of a typical semi-arid steppe in Inner Mongolia, China. *Atmospheric Environment,* 41**,** 5948-5958.

LIU, C., WANG, K., MENG, S., ZHENG, X., ZHOU, Z., HAN, S., CHEN, D. & YANG, Z. 2011a. Effects of irrigation, fertilization and crop straw management on nitrous oxide and nitric oxide emissions from a wheat–maize rotation field in northern China. *Agriculture, Ecosystems & Environment,* 140**,** 226-233.

LIU, Q., YIN, H., CHEN, J., ZHAO, C., CHENG, X., WEI, Y. & LIN, B. 2011b. Belowground responses of Picea asperata seedlings to warming and nitrogen fertilization in the eastern Tibetan Plateau. *Ecological Research,* 26**,** 637-648.

LIVESLEY, S. J., KIESE, R., MIEHLE, P., WESTON, C. J., BUTTERBACH-BAHL, K. & ARNDT, S. K. 2009. Soil-atmosphere exchange of greenhouse gases in a Eucalyptus marginata woodland, a clover-grass pasture, and Pinus radiata and Eucalyptus globulus plantations. *Global Change Biology,* 15**,** 425-440.

LOISEAU, P., CARRÈRE, P., LAFARGE, M., DELPY, R. & DUBLANCHET, J. 2001. Effect of soil-N and urine-N on nitrate leaching under pure grass, pure clover and mixed grass/clover swards. *European Journal of Agronomy,* 14**,** 113-121.

LOREAU, M. & HECTOR, A. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature,* 412**,** 72-76.

LU, M., ZHOU, X., YANG, Q., LI, H., LUO, Y., FANG, C., CHEN, J., YANG, X. & LI, B. 2013. Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. *Ecology,* 94**,** 726-738.

LU, X. Y., CHENG, G. W., XIAO, F. P. & HUO, C. F. 2008. Simulating carbon sequestration and GHGs emissions in Abies fabric forest on the Gongga Mountains using a biogeochemical process model Forest-DNDC. *Journal of Mountain Science,* 5**,** 249-256.

LU, Y. & CONRAD, R. 2005. In Situ Stable Isotope Probing of Methanogenic Archaea in the Rice Rhizosphere. *Science,* 309**,** 1088-1090.

LUO, C., XU, G., CHAO, Z., WANG, S., LIN, X., HU, Y., ZHANG, Z., DUAN, J., CHANG, X., SU, A., LI, Y., ZHAO, X., DU, M., TANG, Y. & KIMBALL, B. 2010. Effect of warming and grazing on litter mass loss and temperature sensitivity of litter and dung mass loss on the Tibetan plateau. *Global Change Biology,* 16**,** 1606-1617.

LUO, Y. Q., WAN, S. Q., HUI, D. F. & WALLACE, L. L. 2001. Acclimatization of soil respiration to warming in a tall grass prairie. *Nature,* 413**,** 622-625.

LUTZ, W. & KC, S. 2010. Dimensions of global population projections: what do we know about future population trends and structures? *Philosophical Transactions of the Royal Society B: Biological Sciences,* 365**,** 2779-2791.

MA, Z., LIU, H., MI, Z., ZHANG, Z., WANG, Y., XU, W., JIANG, L. & HE, J.-S. 2017. Climate warming reduces the temporal stability of plant community biomass production. 8**,** 15378.

MAIRE, V., GROSS, N., DA SILVEIRA PONTES, L., PICON-COCHARD, C. & SOUSSANA, J.-F. 2009. Trade-off between root nitrogen acquisition and shoot nitrogen utilization across 13 co-occurring pasture grass species. *Functional Ecology,* 23**,** 668-679.

MANNING, P., DE VRIES, F. T., TALLOWIN, J. R. B., SMITH, R., MORTIMER, S. R., PILGRIM, E. S., HARRISON, K. A., WRIGHT, D. G., QUIRK, H., BENSON, J., SHIPLEY, B., CORNELISSEN, J. H. C., KATTGE, J., BÖNISCH, G., WIRTH, C. & BARDGETT, R. D. 2015. Simple measures of climate, soil properties and plant traits predict national-scale grassland soil carbon stocks. *Journal of Applied Ecology,* 52**,** 1188-1196.

MARION, G. M., HENRY, G. H. R., FRECKMAN, D. W., JOHNSTONE, J., JONES, G., JONES, M. H., LEVESQUE, E., MOLAU, U., MOLGAARD, P., PARSONS, A. N., SVOBODA, J. & VIRGINIA, R. A. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology,* 3**,** 20-32.

MAWDSLEY, J. L. & BARDGETT, R. D. 1997. Continuous defoliation of perennial ryegrass (Lolium perenne) and white clover (Trifolium repens) and associated changes in the composition and activity of the microbial population of an upland grassland soil. *Biology and Fertility of Soils,* 24**,** 52-58.

MCELROY, M. S., PAPADOPOULOS, Y. A., GLOVER, K. E., DONG, Z., FILLMORE, S. A. E. & JOHNSTON, M. O. 2017. Interactions between cultivars of legume species (Trifolium pratense L., Medicago sativa L.) and grasses (Phleum pratense L., Lolium perenne L.) under different nitrogen levels. *Canadian Journal of Plant Science,* 97**,** 214-225.

MCSHERRY, M. E. & RITCHIE, M. E. 2013. Effects of grazing on grassland soil carbon: a global review. *Glob Chang Biol,* 19**,** 1347-57.

MCSWINEY, C. P. & ROBERTSON, G. P. 2005. Nonlinear response of N2O flux to incremental fertilizer addition in a continuous maize (Zea mays L.) cropping system. *Global Change Biology,* 11**,** 1712-1719.

MELILLO, J. M., BUTLER, S., JOHNSON, J., MOHAN, J., STEUDLER, P., LUX, H., BURROWS, E., BOWLES, F., SMITH, R., SCOTT, L., VARIO, C., HILL, T., BURTON, A., ZHOU, Y. M. & TANG, J. 2011. Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America,* 108**,** 9508-9512.

MELILLO, J. M., STEUDLER, P. A., ABER, J. D., NEWKIRK, K., LUX, H., BOWLES, F. P., CATRICALA, C., MAGILL, A., AHRENS, T. & MORRISSEAU, S. 2002. Soil warming and carbon-cycle feedbacks to the climate system. *Science,* 298**,** 2173-2176.

MILCHUNAS, D. G. 2009. Estimating Root Production: Comparison of 11 Methods in Shortgrass Steppe and Review of Biases. *Ecosystems,* 12**,** 1381-1402.

MISSELBROOK, T. H., VAN DER WEERDEN, T. J., PAIN, B. F., JARVIS, S. C., CHAMBERS, B. J., SMITH, K. A., PHILLIPS, V. R. & DEMMERS, T. G. M. 2000. Ammonia emission factors for UK agriculture. *Atmospheric Environment,* 34**,** 871-880.

MOHAN, J. E., COWDEN, C. C., BAAS, P., DAWADI, A., FRANKSON, P. T., HELMICK, K., HUGHES, E., KHAN, S., LANG, A., MACHMULLER, M., TAYLOR, M. & WITT, C. A. 2014. Mycorrhizal fungi mediation of terrestrial ecosystem responses to global change: mini-review. *Fungal Ecology,* 10**,** 3-19.

MOLINA-HERRERA, S., HAAS, E., KLATT, S., KRAUS, D., AUGUSTIN, J., MAGLIULO, V., TALLEC, T., CESCHIA, E., AMMANN, C., LOUBET, B.,

SKIBA, U., JONES, S., BRÜMMER, C., BUTTERBACH-BAHL, K. & KIESE, R. 2016. A modeling study on mitigation of N2O emissions and NO3 leaching at different agricultural sites across Europe using LandscapeDNDC. *Science of The Total Environment,* 553**,** 128-140.

MORTENSON, M. C., SCHUMAN*, G. E. & INGRAM, L. J. 2004. Carbon Sequestration in Rangelands Interseeded with Yellow-Flowering Alfalfa (Medicago sativa ssp. falcata). *Environmental Management,* 33**,** S475-S481.

MOSIER, A. R., PARTON, W. J., VALENTINE, D. W., OJIMA, D. S., SCHIMEL, D. S. & DELGADO, J. A. 1996. CH4and N2O fluxes in the Colorado shortgrass steppe: 1. Impact of landscape and nitrogen addition. *Global Biogeochemical Cycles,* 10**,** 387-399.

MOYANO, F., KUTSCH, W. & SCHULZE, E. 2007. Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. *Soil Biology and Biochemistry,* 39**,** 843-853.

MUELLER, K. E., HOBBIE, S. E., TILMAN, D. & REICH, P. B. 2013. Effects of plant diversity, N fertilization, and elevated carbon dioxide on grassland soil N cycling in a long-term experiment. *Glob Chang Biol,* 19**,** 1249-61.

MULDER, C. P. H., JUMPPONEN, A., HOGBERG, P. & HUSS-DANELL, K. 2002. How plant diversity and legumes affect nitrogen dynamics in experimental grassland communities. *Oecologia,* 133**,** 412-421.

NAKANO-HYLANDER, A. & OLSSON, P. A. 2007. Carbon allocation in mycelia of arbuscular mycorrhizal fungi during colonisation of plant seedlings. *Soil Biology and Biochemistry,* 39**,** 1450-1458.

NEAL, C., SKEFFINGTON, R., NEAL, M., WYATT, R., WICKHAM, H., HILL, L. & HEWITT, N. 2004. Rainfall and runoff water quality of the Pang and Lambourn, tributaries of the River Thames, south-eastern England. *Hydrology and Earth System Sciences,* 8**,** 601-613.

NEIL 1992. Comparison of Soil Coring and Ingrowth Methods for Measuring Belowground Production

NGUYEN, C. 2003. Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie,* 23**,** 375-396.

NIBOYET, A., BROWN, J. R., DIJKSTRA, P., BLANKINSHIP, J. C., LEADLEY, P. W., LE ROUX, X., BARTHES, L., BARNARD, R. L., FIELD, C. B. & HUNGATE, B. A. 2011. Global Change Could Amplify Fire Effects on Soil Greenhouse Gas Emissions. *PLOS ONE,* 6**,** e20105.

NIKLAUS, P., WARDLE, D. & TATE, K. 2006. Effects of Plant Species Diversity and Composition on Nitrogen Cycling and the Trace Gas Balance of Soils. *Plant and Soil,* 282**,** 83-98.

NIKLAUS, P. A., LE ROUX, X., POLY, F., BUCHMANN, N., SCHERER-LORENZEN, M., WEIGELT, A. & BARNARD, R. L. 2016. Plant species diversity affects soil-atmosphere fluxes of methane and nitrous oxide. *Oecologia,* 181**,** 919-30.

NIKLIŃSKA, M., MARYAŃSKI, M. & LASKOWSKI, R. 1999. Effect of temperature on humus respiration rate and nitrogen mineralization: Implications for global climate change. *Biogeochemistry,* 44**,** 239-257.

NOTTINGHAM, A. T., TURNER, B. L., WINTER, K., VAN DER HEIJDEN, M. G. & TANNER, E. V. 2010. Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. *New Phytol,* 186**,** 957-67.

NYFELER, D., HUGUENIN-ELIE, O., SUTER, M., FROSSARD, E., CONNOLLY, J. & LUSCHER, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology,* 46**,** 683-691.

NYFELER, D., HUGUENIN-ELIE, O., SUTER, M., FROSSARD, E. & LÜSCHER, A. 2011. Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems & Environment,* 140**,** 155-163.

O'NEILL, B. C., OPPENHEIMER, M., WARREN, R., HALLEGATTE, S., KOPP, R. E., PORTNER, H. O., SCHOLES, R., BIRKMANN, J., FODEN, W., LICKER, R., MACH, K. J., MARBAIX, P., MASTRANDREA, M. D., PRICE, J., TAKAHASHI, K., VAN YPERSELE, J. P. & YOHE, G. 2017. IPCC reasons for concern regarding climate change risks. *Nature Climate Change,* 7**,** 28-37.

OBERSON, A., FROSSARD, E., BÜHLMANN, C., MAYER, J., MÄDER, P. & LÜSCHER, A. 2013. Nitrogen fixation and transfer in grass-clover leys under organic and conventional cropping systems. *Plant and Soil,* 371**,** 237-255.

OECHEL, W. C., VOURLITIS, G. L., HASTINGS, S. J., ZULUETA, R. C., HINZMAN, L. & KANE, D. 2000. Acclimation of ecosystem CO2 exchange in the Alaskan Arctic in response to decadal climate warming. *Nature,* 406**,** 978-981.

OERTEL, C., MATSCHULLAT, J., ZURBA, K., ZIMMERMANN, F. & ERASMI, S. 2016. Greenhouse gas emissions from soils—A review. *Chemie der Erde - Geochemistry,* 76**,** 327-352.

OKSANEN, J., GUILLAUME BLANCHET, F., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P. R., O'HARA, R. B., SIMPSON, G. L., SOLYMOS, P., STEVENS, M. H. H., SZOECS, E. & WAGNER, H. 2017. vegan: Community Ecology Package.

OLESEN, J. E. & BINDI, M. 2002. Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy,* 16**,** 239-262.

ORWIN, K. H., BUCKLAND, S. M., JOHNSON, D., TURNER, B. L., SMART, S., OAKLEY, S. & BARDGETT, R. D. 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of Ecology,* 98**,** 1074-1083.

OSTLE, N. J., LEVY, P. E., EVANS, C. D. & SMITH, P. 2009a. UK land use and soil carbon sequestration. *Land Use Policy,* 26**,** S274-S283.

OSTLE, N. J., SMITH, P., FISHER, R., IAN WOODWARD, F., FISHER, J. B., SMITH, J. U., GALBRAITH, D., LEVY, P., MEIR, P., MCNAMARA, N. P. & BARDGETT, R. D. 2009b. Integrating plant-soil interactions into global carbon cycle models. *Journal of Ecology,* 97**,** 851-863.

OSTONEN, I., PÜTTSEPP, Ü., BIEL, C., ALBERTON, O., BAKKER, M. R., LÕHMUS, K., MAJDI, H., METCALFE, D., OLSTHOORN, A. F. M., PRONK, A., VANGUELOVA, E., WEIH, M. & BRUNNER, I. 2007. Specific root length as an indicator of environmental change. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology,* 141**,** 426-442.

PATERSON 2003. Importance ofrhizodeposition in the coupling ofplant andmicrobial productivity

PAYNEL, F., MURRAY, P. J. & CLIQUET, J. B. 2001. Root exudates: a pathway for short-term N transfer from clover and ryegrass. *Plant and Soil,* 229**,** 235-243.

PENDALL, E., BRIDGHAM, S., HANSON, P. J., HUNGATE, B., KICKLIGHTER, D. W., JOHNSON, D. W., LAW, B. E., LUO, Y. Q., MEGONIGAL, J. P., OLSRUD, M., RYAN, M. G. & WAN, S. Q. 2004. Below-ground process responses to elevated CO2 and temperature: a discussion of observations, measurement methods, and models. *New Phytologist,* 162**,** 311-322.

PENG, F., YOU, Q. G., XU, M. H., ZHOU, X. H., WANG, T., GUO, J. & XUE, X. 2015. Effects of experimental warming on soil respiration and its components in an alpine meadow in the permafrost region of the Qinghai-Tibet Plateau. *European Journal of Soil Science,* 66**,** 145-154.

PERSONENI, E. & LOISEAU, P. 2005. Species strategy and N fluxes in grassland soil. *European Journal of Agronomy,* 22**,** 217-229.

PETZ, K., ALKEMADE, R., BAKKENES, M., SCHULP, C. J. E., VAN DER VELDE, M. & LEEMANS, R. 2014. Mapping and modelling trade-offs and synergies between grazing intensity and ecosystem services in rangelands using global-scale datasets and models. *Global Environmental Change,* 29**,** 223-234.

PHILLIPS, R. L., WHALEN, S. C. & SCHLESINGER, W. H. 2001. Influence of atmospheric CO2 enrichment on methane consumption in a temperate forest soil. *Global Change Biology,* 7**,** 557-563.

PILON, R., PICON-COCHARD, C., BLOOR, J. M. G., REVAILLOT, S., KUHN, E., FALCIMAGNE, R., BALANDIER, P. & SOUSSANA, J.-F. 2013. Grassland root demography responses to multiple climate change drivers depend on root morphology. *Plant and Soil,* 364**,** 395-408.

PINHEIRO, J., BATES, D., DEBROY, S., SARKAR, D. & TEAM, R. C. 2017. nlme: Linear and Nonlinear Mixed Effects Models.

PIRHOFER-WALZL, K., RASMUSSEN, J., HØGH-JENSEN, H., ERIKSEN, J., SØEGAARD, K. & RASMUSSEN, J. 2012. Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. *Plant and Soil,* 350**,** 71-84.

POORTER, H., VAN DER WERF, A., ATKIN, O. K. & LAMBERS, H. 1991. Respiratory energy requirements of roots vary with the potential growth rate of a plant species. *Physiologia Plantarum,* 83**,** 469-475.

POST, E., FORCHHAMMER, M. C., BRET-HARTE, M. S., CALLAGHAN, T. V., CHRISTENSEN, T. R., ELBERLING, B., FOX, A. D., GILG, O., HIK, D. S., HOYE, T. T., IMS, R. A., JEPPESEN, E., KLEIN, D. R., MADSEN, J., MCGUIRE, A. D., RYSGAARD, S., SCHINDLER, D. E., STIRLING, I., TAMSTORF, M. P., TYLER, N. J. C., VAN DER WAL, R., WELKER, J., WOOKEY, P. A., SCHMIDT, N. M. & AASTRUP, P. 2009. Ecological Dynamics Across the Arctic Associated with Recent Climate Change. *Science,* 325**,** 1355-1358.

POWLSON, D. S., GOULDING, K. W. T., WILLISON, T. W., WEBSTER, C. P. & HÜTSCH, B. W. 1997. The effect of agriculture on methane oxidation in soil. *Nutrient Cycling in Agroecosystems,* 49**,** 59-70.

R DEVELOPMENT CORE TEAM 2017. R: A language and environment to statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

RAFIQUE, R., ANEX, R., HENNESSY, D. & KIELY, G. 2012. What are the impacts of grazing and cutting events on the N2O dynamics in humid temperate grassland? *Geoderma,* 181**,** 36-44.

RAFIQUE, R., HENNESSY, D. & KIELY, G. 2011a. Nitrous Oxide Emission from Grazed Grassland Under Different Management Systems. *Ecosystems,* 14**,** 563-582.

RAFIQUE, R., PEICHL, M., HENNESSY, D. & KIELY, G. 2011b. Evaluating management effects on nitrous oxide emissions from grasslands using the process-based DeNitrification–DeComposition (DNDC) model. *Atmospheric Environment,* 45**,** 6029-6039.

RAIESI, F. & ASADI, E. 2006. Soil microbial activity and litter turnover in native grazed and ungrazed rangelands in a semiarid ecosystem. *Biology and Fertility of Soils,* 43**,** 76-82.

READ, D., BEERLING, D., CANNELL, M., COX, P., CURRAN, P., GRACE, J., INESON, P., JARVIS, P., MALHI, Y., POWLSON, D., SHEPHERD, J. & WOODWARD, I. 2001. The role of land carbon sinks in mitigating global climate change. London, UK: Royal Society.

REES, R. M., AUGUSTIN, J., ALBERTI, G., BALL, B. C., BOECKX, P., CANTAREL, A., CASTALDI, S., CHIRINDA, N., CHOJNICKI, B., GIEBELS, M., GORDON, H., GROSZ, B., HORVATH, L., JUSZCZAK, R., KASIMIR KLEMEDTSSON, Å., KLEMEDTSSON, L., MEDINETS, S., MACHON, A., MAPANDA, F., NYAMANGARA, J., OLESEN, J. E., REAY, D. S., SANCHEZ, L., SANZ COBENA, A., SMITH, K. A., SOWERBY, A., SOMMER, M., SOUSSANA, J. F., STENBERG, M., TOPP, C. F. E., VAN CLEEMPUT, O., VALLEJO, A., WATSON, C. A. & WUTA, M. 2013. Nitrous oxide emissions from European agriculture – an analysis of variability and drivers of emissions from field experiments. *Biogeosciences,* 10**,** 2671-2682.

REICH, P. B., HUNGATE, B. A. & LUO, Y. Q. 2006. Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. *Annual Review of Ecology Evolution and Systematics,* 37**,** 611-636.

RILLIG, M. C. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters,* 7**,** 740-754.

RILLIG, M. C. & MUMMEY, D. L. 2006. Mycorrhizas and soil structure. *New Phytologist,* 171**,** 41-53.

RILLIG, M. C., TRESEDER, K. K. & ALLEN, M. F. 2002a. Global Change and Mycorrhizal Fungi. *In:* M.G.A., V. D. H. & (EDS), S. I. R. (eds.) *Mycorrhizal Ecology. Ecological Studies (Analysis and Synthesis).* Berlin, Heidelberg: Springer, Berlin, Heidelberg.

RILLIG, M. C., WRIGHT, S. F., SHAW, M. R. & FIELD, C. B. 2002b. Artificial climate warming positively affects arbuscular mycorrhizae but decreases soil aggregate water stability in an annual grassland. *Oikos,* 97**,** 52-58.

RIME, T. & NIKLAUS, P. A. 2017. Spatio-temporal dynamics of soil CH4 uptake after application of N fertilizer with and without the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). *Soil Biology & Biochemistry,* 104**,** 218-225.

ROCHETTE, P. & JANZEN, H. H. 2005. Towards a revised coefficient for estimating N2O emissions from legumes. *Nutrient Cycling in Agroecosystems,* 73**,** 171-179.

RUSER, R., FLESSA, H., RUSSOW, R., SCHMIDT, G., BUEGGER, F. & MUNCH, J. C. 2006. Emission of N2O, N2 and CO2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biology and Biochemistry,* 38**,** 263-274.

RUSTAD, L. E., CAMPBELL, J. L., MARION, G. M., NORBY, R. J., MITCHELL, M. J., HARTLEY, A. E., CORNELISSEN, J. H. C., GUREVITCH, J. & GCTE-NEWS 2001. A meta-analysis of the response of soil respiration, net nitrogen

mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia,* 126**,** 543-562.

RUSTAD, L. E. & FERNANDEZ, I. J. 1998. Experimental soil warming effects on CO2 and CH4 flux from a low elevation spruce–fir forest soil in Maine, USA. *Global Change Biology,* 4**,** 597-605.

RYAN, M. G. & LAW, B. E. 2005. Interpreting, measuring, and modeling soil respiration. *Biogeochemistry,* 73**,** 3-27.

SAGGAR, S., GILTRAP, D. L., LI, C. & TATE, K. R. 2007a. Modelling nitrous oxide emissions from grazed grasslands in New Zealand. *Agriculture, Ecosystems & Environment,* 119**,** 205-216.

SAGGAR, S., HEDLEY, C. B., GILTRAP, D. L. & LAMBIE, S. M. 2007b. Measured and modelled estimates of nitrous oxide emission and methane consumption from a sheep-grazed pasture. *Agriculture, Ecosystems & Environment,* 122**,** 357-365.

SAGGAR, S., JHA, N., DESLIPPE, J., BOLAN, N. S., LUO, J., GILTRAP, D. L., KIM, D. G., ZAMAN, M. & TILLMAN, R. W. 2013. Denitrification and N2O:N2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. *Sci Total Environ,* 465**,** 173-95.

SAGGAR, S., LUO, J., GILTRAP, D. L. & MADDENA, M. 2009. Nitrous oxide emission from temperate grasslands: process, measurements, modelling and mitigation. *In:* SHELDON, A. I. & BARNHART, E. P. (eds.) *Nitrous oxide emissions research progress.* New York: Nova Science Publishers.

SCHELDE, K., CELLIER, P., BERTOLINI, T., DALGAARD, T., WEIDINGER, T., THEOBALD, M. R. & OLESEN, J. E. 2012. Spatial and temporal variability of nitrous oxide emissions in a mixed farming landscape of Denmark. *Biogeosciences,* 9**,** 2989-3002.

SCHEURWATER, I., DUNNEBACKE, M., EISING, R. & LAMBERS, H. 2000. Respiratory costs and rate of protein turnover in the roots of a fast-growing (Dactylis glomerata L.) and a slow-growing (Festuca ovina L.) grass species. *J Exp Bot,* 51**,** 1089-97.

SCHLESINGER, W. H. & ANDREWS, J. A. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry,* 48**,** 7-20.

SCHMID, M., NEFTEL, A., RIEDO, M. & FUHRER, J. 2001. Process-based modelling of nitrous oxide emissions from different nitrogen sources in mown grassland. *Nutrient Cycling in Agroecosystems,* 60**,** 177-187.

SELBIE, D. R., LANIGAN, G. J., LAUGHLIN, R. J., DI, H. J., MOIR, J. L., CAMERON, K. C., CLOUGH, T. J., WATSON, C. J., GRANT, J., SOMERS, C. & RICHARDS, K. G. 2015. Confirmation of co-denitrification in grazed grassland. *Scientific Reports,* 5.

SHCHERBAK, I., MILLAR, N. & ROBERTSON, G. P. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences of the United States of America,* 111**,** 9199-9204.

SIX, J., BOSSUYT, H., DEGRYZE, S. & DENEF, K. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research,* 79**,** 7-31.

SKIBA, U. & SMITH, K. A. 2000. The control of nitrous oxide emissions from agricultural and natural soils. *Chemosphere - Global Change Science,* 2**,** 379-386.

SMITH, K. A., BALL, T., CONEN, F., DOBBIE, K. E., MASSHEDER, J. & REY, A. 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science,* 54**,** 779-791.

SMITH, P., DAVIES, C. A., OGLE, S., ZANCHI, G., BELLARBY, J., BIRD, N., BODDEY, R. M., MCNAMARA, N. P., POWLSON, D., COWIE, A., VAN NOORDWIJK, M., DAVIS, S. C., RICHTER, D. D. E. B., KRYZANOWSKI, L., VAN WIJK, M. T., STUART, J., KIRTON, A., EGGAR, D., NEWTON-CROSS, G., ADHYA, T. K. & BRAIMOH, A. K. 2012. Towards an integrated global framework to assess the impacts of land use and management change on soil carbon: current capability and future vision. *Global Change Biology,* 18**,** 2089-2101.

SMITH, P., HOUSE, J. I., BUSTAMANTE, M., SOBOCKÁ, J., HARPER, R., PAN, G., WEST, P. C., CLARK, J. M., ADHYA, T., RUMPEL, C., PAUSTIAN, K., KUIKMAN, P., COTRUFO, M. F., ELLIOTT, J. A., MCDOWELL, R., GRIFFITHS, R. I., ASAKAWA, S., BONDEAU, A., JAIN, A. K., MEERSMANS, J. & PUGH, T. A. M. 2016. Global change pressures on soils from land use and management. *Global Change Biology,* 22**,** 1008-1028.

SMITH, P., MARTINO, D., CAI, Z., GWARY, D., JANZEN, H., KUMAR, P., MCCARL, B., OGLE, S., O'MARA, F., RICE, C., SCHOLES, B., SIROTENKO, O., HOWDEN, M., MCALLISTER, T., PAN, G., ROMANENKOV, V., SCHNEIDER, U., TOWPRAYOON, S., WATTENBACH, M. & SMITH, J. 2008a. Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 363**,** 789-813.

SMITH, P., SMITH, J. U., POWLSON, D. S., MCGILL, W. B., ARAH, J. R. M., CHERTOV, O. G., COLEMAN, K., FRANKO, U., FROLKING, S., JENKINSON, D. S., JENSEN, L. S., KELLY, R. H., KLEIN-GUNNEWIEK, H., KOMAROV, A. S., LI, C., MOLINA, J. A. E., MUELLER, T., PARTON, W. J., THORNLEY, J. H. M. & WHITMORE, A. P. 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. *Geoderma,* 81**,** 153-225.

SMITH, R. S., SHIEL, R. S., BARDGETT, R. D., MILLWARD, D., CORKHILL, P., EVANS, P., QUIRK, H., HOBBS, P. J. & KOMETA, S. T. 2008b. Long-term change in vegetation and soil microbial communities during the phased restoration of traditional meadow grassland. *Journal of Applied Ecology,* 45**,** 670-679.

SMITH, S. E. & READ, D. 2008. *Mycorrhizal Symbiosis,* London, Academic Press.

SMITH, W. N., GRANT, B. B., DESJARDINS, R. L., WORTH, D., LI, C., BOLES, S. H. & HUFFMAN, E. C. 2010. A tool to link agricultural activity data with the DNDC model to estimate GHG emission factors in Canada. *Agriculture, Ecosystems & Environment,* 136**,** 301-309.

SONNEMANN, I., BUCHHOLZ, Y., RAUTENBERG, D. & WURST, S. 2016. Land use intensity modulates the impact of root herbivores on plant interactions with above- and below-ground organisms. *Agriculture, Ecosystems & Environment,* 219**,** 26-31.

SOUDZILOVSKAIA, N. A., ELUMEEVA, T. G., ONIPCHENKO, V. G., SHIDAKOV, II, SALPAGAROVA, F. S., KHUBIEV, A. B., TEKEEV, D. K. & CORNELISSEN, J. H. 2013. Functional traits predict relationship between

plant abundance dynamic and long-term climate warming. *Proc Natl Acad Sci U S A,* 110**,** 18180-4.

SOUSSANA, J. F., ALLARD, V., PILEGAARD, K., AMBUS, P., AMMAN, C., CAMPBELL, C., CESCHIA, E., CLIFTON-BROWN, J., CZOBEL, S., DOMINGUES, R., FLECHARD, C., FUHRER, J., HENSEN, A., HORVATH, L., JONES, M., KASPER, G., MARTIN, C., NAGY, Z., NEFTEL, A., RASCHI, A., BARONTI, S., REES, R. M., SKIBA, U., STEFANI, P., MANCA, G., SUTTON, M., TUBA, Z. & VALENTINI, R. 2007. Full accounting of the greenhouse gas (CO2, N2O, CH4) budget of nine European grassland sites. *Agriculture, Ecosystems & Environment,* 121**,** 121-134.

SPEHN, E. M., HECTOR, A., JOSHI, J., SCHERER-LORENZEN, M., SCHMID, B., BAZELEY-WHITE, E., BEIERKUHNLEIN, C., CALDEIRA, M. C., DIEMER, M., DIMITRAKOPOULOS, P. G., FINN, J. A., FREITAS, H., GILLER, P. S., GOOD, J., HARRIS, R., HÖGBERG, P., HUSS-DANELL, K., JUMPPONEN, A., KORICHEVA, J., LEADLEY, P. W., LOREAU, M., MINNS, A., MULDER, C. P. H., O'DONOVAN, G., OTWAY, S. J., PALMBORG, C., PEREIRA, J. S., PFISTERER, A. B., PRINZ, A., READ, D. J., SCHULZE, E. D., SIAMANTZIOURAS, A. S. D., TERRY, A. C., TROUMBIS, A. Y., WOODWARD, F. I., YACHI, S. & LAWTON, J. H. 2005. ECOSYSTEM EFFECTS OF BIODIVERSITY MANIPULATIONS IN EUROPEAN GRASSLANDS. *Ecological Monographs,* 75**,** 37-63.

SPEHN, E. M., JOSHI, J., SCHMID, B., DIEMER, M. & KORNER, C. 2000. Above-ground resource use increases with plant species richness in experimental grassland ecosystems. *Functional Ecology,* 14**,** 326-337.

SPEHN, E. M., SCHERER-LORENZEN, M., SCHMID, B., HECTOR, A., CALDEIRA, M. C., DIMITRAKOPOULOS, P. G., FINN, J. A., JUMPPONEN, A., O'DONNOVAN, G., PEREIRA, J. S., SCHULZE, E. D., TROUMBIS, A. Y. & KORNER, C. 2002. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. *Oikos,* 98**,** 205-218.

SPOTT, O., RUSSOW, R. & STANGE, C. F. 2011. Formation of hybrid N2O and hybrid N2 due to codenitrification: First review of a barely considered process of microbially mediated N-nitrosation. *Soil Biology and Biochemistry,* 43**,** 1995-2011.

STADDON, P. L., HEINEMEYER, A. & FITTER, A. H. 2002. Mycorrhizas and global environmental change: research at different scales. *Plant and Soil,* 244**,** 253-261.

STEINKAMP, R., BUTTERBACH-BAHL, K. & PAPEN, H. 2001. Methane oxidation by soils of an N limited and N fertilized spruce forest in the Black Forest, Germany. *Soil Biology & Biochemistry,* 33**,** 145-153.

STEVENS, R. J., LAUGHLIN, R. J., BURNS, L. C., ARAH, J. R. M. & HOOD, R. C. 1997. Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry,* 29**,** 139-151.

SUBKE, J.-A., HAHN, V., BATTIPAGLIA, G., LINDER, S., BUCHMANN, N. & COTRUFO, M. F. 2004. Feedback interactions between needle litter decomposition and rhizosphere activity. *Oecologia,* 139**,** 551-559.

SUN, H., ZHANG, C., SONG, C., CHANG, S. X., GU, B., CHEN, Z., PENG, C., CHANG, J. & GE, Y. 2013. The effects of plant diversity on nitrous oxide emissions in hydroponic microcosms. *Atmospheric Environment,* 77**,** 544-547.

SUTER, M., CONNOLLY, J., FINN, J. A., LOGES, R., KIRWAN, L., SEBASTIÀ, M.-T. & LÜSCHER, A. 2015. Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Global Change Biology,* 21**,** 2424-2438.

SUWANWAREE, P. & ROBERTSON, G. P. 2005. Methane Oxidation in Forest, Successional, and No-till Agricultural Ecosystems. *Soil Science Society of America Journal,* 69**,** 1722-1729.

SYAKILA, A. & KROEZE, C. 2011. The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management,* 1**,** 17-26.

TANG, X.-L., ZHOU, G.-Y., LIU, S.-G., ZHANG, D.-Q., LIU, S.-Z., LI, J. & ZHOU, C.-Y. 2006. Dependence of Soil Respiration on Soil Temperature and Soil Moisture in Successional Forests in Southern China. *Journal of Integrative Plant Biology,* 48**,** 654-663.

TAUBE, F., GIERUS, M., HERMANN, A., LOGES, R. & SCHÖNBACH, P. 2014. Grassland and globalization – challenges for north-west European grass and forage research. *Grass and Forage Science,* 69**,** 2-16.

THOMSON, A. J., GIANNOPOULOS, G., PRETTY, J., BAGGS, E. M. & RICHARDSON, D. J. 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society B-Biological Sciences,* 367**,** 1157-1168.

THOMSON, B. C., OSTLE, N. J., MCNAMARA, N. P., OAKLEY, S., WHITELEY, A. S., BAILEY, M. J. & GRIFFITHS, R. I. 2013. Plant soil interactions alter carbon cycling in an upland grassland soil. *Frontiers in Microbiology,* 4.

TIAN, H., CHEN, G., LU, C., XU, X., REN, W., ZHANG, B., BANGER, K., TAO, B., PAN, S., LIU, M., ZHANG, C., BRUHWILER, L. & WOFSY, S. 2015. Global methane and nitrous oxide emissions from terrestrial ecosystems due to multiple environmental changes. *Ecosystem Health and Sustainability,* 1**,** 1-20.

TILMAN, D., REICH, P. B., KNOPS, J., WEDIN, D., MIELKE, T. & LEHMAN, C. 2001. Diversity and productivity in a long-term grassland experiment. *Science,* 294**,** 843-845.

TISDALL, J. M. & OADES, J. M. 1982. Organic matter and water-stable aggregates in soils. *Journal of Soil Science,* 33**,** 141-163.

TOPP, E. & PATTEY, E. 1997. Soils as sources and sinks for atmospheric methane. *Canadian Journal of Soil Science,* 77**,** 167-178.

TORN, M. S. & HARTE, J. 1996. Methane consumption by montane soils: implications for positive and negative feedback with climatic change. *Biogeochemistry,* 32**,** 53-67.

TRESEDER, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecol Lett,* 11**,** 1111-20.

TRESEDER, K. K. & ALLEN, M. F. 2000. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO2 and nitrogen deposition. *New Phytologist,* 147**,** 189-200.

TRUMBORE, S. 2000. AGE OF SOIL ORGANIC MATTER AND SOIL RESPIRATION: RADIOCARBON CONSTRAINTS ON BELOWGROUND C DYNAMICS. *Ecological Applications,* 10**,** 399-411.

TRUMBORE, S. 2006. Carbon respired by terrestrial ecosystems – recent progress and challenges. *Global Change Biology,* 12**,** 141-153.

TRUMBORE, S., DA COSTA, E. S., NEPSTAD, D. C., DE CAMARGO, P. B., MARTINELLI, L. I. Z. A., RAY, D., RESTOM, T. & SILVER, W. 2006.

Dynamics of fine root carbon in Amazonian tropical ecosystems and the contribution of roots to soil respiration. *Global Change Biology,* 12**,** 217-229.
TU, L. H., HU, T. X., ZHANG, J., LI, X. W., HU, H. L., LIU, L. & XIAO, Y. L. 2013. Nitrogen addition stimulates different components of soil respiration in a subtropical bamboo ecosystem. *Soil Biology & Biochemistry,* 58**,** 255-264.
TURNER, M. M. & HENRY, H. A. 2010. Net nitrogen mineralization and leaching in response to warming and nitrogen deposition in a temperate old field: the importance of winter temperature. *Oecologia,* 162**,** 227-36.
USSIRI, D. & LAL, R. 2012. *Soil Emission of Nitrous Oxide and its Mitigation,* Springer Netherlands.
VAN DER KRIFT, T. A. J. & BERENDSE, F. 2001. The effect of plant species on soil nitrogen mineralization. *Journal of Ecology,* 89**,** 555-561.
VAN DER KRIFT, T. A. J., GIOACCHINI, P., KUIKMAN, P. J. & BERENDSE, F. 2001. Effects of high and low fertility plant species on dead root decomposition and nitrogen mineralisation. *Soil Biology and Biochemistry,* 33**,** 2115-2124.
VAN GROENIGEN, J. W., HUYGENS, D., BOECKX, P., KUYPER, T. W., LUBBERS, I. M., RÜTTING, T. & GROFFMAN, P. M. 2015. The soil N cycle: new insights and key challenges. *SOIL,* 1**,** 235-256.
VAN GROENIGEN, K. J., SIX, J., HUNGATE, B. A., DE GRAAFF, M. A., VAN BREEMEN, N. & VAN KESSEL, C. 2006. Element interactions limit soil carbon storage. *Proceedings of the National Academy of Sciences of the United States of America,* 103**,** 6571-6574.
VAN RUIJVEN, J. & BERENDSE, F. 2005. Diversity-productivity relationships: initial effects, long-term patterns, and underlying mechanisms. *Proc Natl Acad Sci U S A,* 102**,** 695-700.
VARGAS, R., BALDOCCHI, D. D., QUEREJETA, J. I., CURTIS, P. S., HASSELQUIST, N. J., JANSSENS, I. A., ALLEN, M. F. & MONTAGNANI, L. 2010. Ecosystem CO2 fluxes of arbuscular and ectomycorrhizal dominated vegetation types are differentially influenced by precipitation and temperature. *New Phytologist,* 185**,** 226-236.
VERESOGLOU, S. D., CHEN, B. & RILLIG, M. C. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biology and Biochemistry,* 46**,** 53-62.
WALKER, T. N., GARNETT, M. H., WARD, S. E., OAKLEY, S., BARDGETT, R. D. & OSTLE, N. J. 2016. Vascular plants promote ancient peatland carbon loss with climate warming. *Glob Chang Biol,* 22**,** 1880-9.
WAN, S., LUO, Y. & WALLACE, L. L. 2002. Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biology,* 8**,** 754-768.
WAN, S. Q. & LUO, Y. Q. 2003. Substrate regulation of soil respiration in a tallgrass prairie: Results of a clipping and shading experiment. *Global Biogeochemical Cycles,* 17.
WANG, B. & QIU, Y. L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza,* 16**,** 299-363.
WANG, C., BUTTERBACH-BAHL, K., HE, N., WANG, Q., XING, X. & HAN, X. 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, H., LIU, H., WANG, Y., XU, W., LIU, A., MA, Z., MI, Z., ZHANG, Z., WANG, S. & HE, J.-S. 2017a. Warm- and cold- season grazing affect soil

respiration differently in alpine grasslands. *Agriculture, Ecosystems & Environment,* 248**,** 136-143.

WANG, Q., ZHANG, W., SUN, T., CHEN, L., PANG, X., WANG, Y. & XIAO, F. 2017b. N and P fertilization reduced soil autotrophic and heterotrophic respiration in a young Cunninghamia lanceolata forest. *Agricultural and Forest Meteorology,* 232**,** 66-73.

WANG, X., LIU, L., PIAO, S., JANSSENS, I. A., TANG, J., LIU, W., CHI, Y., WANG, J. & XU, S. 2014a. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. *Glob Chang Biol,* 20**,** 3229-37.

WANG, X., MCCONKEY, B. G., VANDENBYGAART, A. J., FAN, J., IWAASA, A. & SCHELLENBERG, M. 2016. Grazing improves C and N cycling in the Northern Great Plains: a meta-analysis. 6**,** 33190.

WANG, Y., CHEN, H., ZHU, Q., PENG, C., WU, N., YANG, G., ZHU, D., TIAN, J., TIAN, L., KANG, X., HE, Y., GAO, Y. & ZHAO, X. 2014b. Soil methane uptake by grasslands and forests in China. *Soil Biology and Biochemistry,* 74**,** 70-81.

WANG, Y., HU, Y., JI, B., LIU, G. & XUE, M. 2003. An Investigation on the Relationship Between Emission/Uptake of Greenhouse Gases and Environmental Factors in Serniarid Grassland. *Advances in Atmospheric Sciences,* 20**,** 119-127.

WARD, D., KIRKMAN, K., HAGENAH, N. & TSVUURA, Z. 2017. Soil respiration declines with increasing nitrogen fertilization and is not related to productivity in long-term grassland experiments. *Soil Biology and Biochemistry,* 115**,** 415-422.

WARD, S. E., BARDGETT, R. D., MCNAMARA, N. P. & OSTLE, N. J. 2009. Plant functional group identity influences short-term peatland ecosystem carbon flux: evidence from a plant removal experiment. *Functional Ecology,* 23**,** 454-462.

WARD, S. E., OSTLE, N. J., OAKLEY, S., QUIRK, H., HENRYS, P. A. & BARDGETT, R. D. 2013. Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. *Ecol Lett,* 16**,** 1285-93.

WARD, S. E., SMART, S. M., QUIRK, H., TALLOWIN, J. R., MORTIMER, S. R., SHIEL, R. S., WILBY, A. & BARDGETT, R. D. 2016. Legacy effects of grassland management on soil carbon to depth. *Glob Chang Biol,* 22**,** 2929-38.

WEI, D., XU-RI, TENZIN-TARCHEN, WANG, Y. & WANG, Y. 2015. Considerable methane uptake by alpine grasslands despite the cold climate: in situ measurements on the central Tibetan Plateau, 2008–2013. *Global Change Biology,* 21**,** 777-788.

WEI, L., LIU, J., SU, J., JING, G., ZHAO, J., CHENG, J. & JIN, J. 2016. Effect of clipping on soil respiration components in temperate grassland of Loess Plateau. *European Journal of Soil Biology,* 75**,** 157-167.

WICKHAM, H. 2011. The Split-Apply-Combine Strategy for Data Analysis. *2011,* 40**,** 29.

WMO 2016. Greenhouse gas bulletin: the state of greenhouse gases in the atmosphere based on observations through 2015. Geneva, Switzerland.

WRAGE, N., VELTHOF, G. L., VAN BEUSICHEM, M. L. & OENEMA, O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry,* 33**,** 1723-1732.

WU, Z. T., DIJKSTRA, P., KOCH, G. W. & HUNGATE, B. A. 2012. Biogeochemical and ecological feedbacks in grassland responses to warming. *Nature Climate Change,* 2**,** 458-461.

XIA, J., NIU, S. & WAN, S. 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. *Global Change Biology,* 15**,** 1544-1556.

XU, L., BALDOCCHI, D. D. & TANG, J. 2004. How soil moisture, rain pulses, and growth alter the response of ecosystem respiration to temperature. *Global Biogeochemical Cycles,* 18**,** n/a-n/a.

XUE, X., PENG, F., YOU, Q. G., XU, M. H. & DONG, S. Y. 2015. Belowground carbon responses to experimental warming regulated by soil moisture change in an alpine ecosystem of the Qinghai-Tibet Plateau. *Ecology and Evolution,* 5**,** 4063-4078.

YOSHITAKE, S., TABEI, N., MIZUNO, Y., YOSHIDA, H., SEKINE, Y., TATSUMURA, M. & KOIZUMI, H. 2015. Soil microbial response to experimental warming in cool temperate semi-natural grassland in Japan. *Ecological Research,* 30**,** 235-245.

YU, L. J., HUANG, Y., ZHANG, W., LI, T. T. & SUN, W. J. 2017. Methane uptake in global forest and grassland soils from 1981 to 2010. *Science of the Total Environment,* 607**,** 1163-1172.

YUE, P., LI, K., GONG, Y., HU, Y., MOHAMMAT, A., CHRISTIE, P. & LIU, X. 2016. A five-year study of the impact of nitrogen addition on methane uptake in alpine grassland. 6**,** 32064.

YUSTE, J. C., MA, S. & BALDOCCHI, D. D. 2010. Plant-soil interactions and acclimation to temperature of microbial-mediated soil respiration may affect predictions of soil CO2 efflux. *Biogeochemistry,* 98**,** 127-138.

ZHANG, B., CHEN, S. Y., ZHANG, J. F., HE, X. Y., LIU, W. J., ZHAO, Q., ZHAO, L. & TIAN, C. J. 2015a. Depth-related responses of soil microbial communities to experimental warming in an alpine meadow on the Qinghai-Tibet Plateau. *European Journal of Soil Science,* 66**,** 496-504.

ZHANG, C. P., NIU, D. C., HALL, S. J., WEN, H. Y., LI, X. D., FU, H., WAN, C. G. & ELSER, J. J. 2014a. Effects of simulated nitrogen deposition on soil respiration components and their temperature sensitivities in a semiarid grassland. *Soil Biology & Biochemistry,* 75**,** 113-123.

ZHANG, L., GUO, D., NIU, S., WANG, C., SHAO, C. & LI, L. 2012. Effects of Mowing on Methane Uptake in a Semiarid Grassland in Northern China. *PLOS ONE,* 7**,** e35952.

ZHANG, L., HOU, L., LAANBROEK, H., GUO, D. & WANG, Q. 2015b. Effects of Mowing Heights on N2O Emission from Temperate Grasslands in Inner Mongolia, Northern China. *American Journal of Climate Change,* 4**,** 397-407.

ZHANG, L. H., HOU, L. Y., GUO, D. F., LI, L. H. & XU, X. F. 2017. Interactive impacts of nitrogen input and water amendment on growing season fluxes of CO2, CH4, and N2O in a semiarid grassland, Northern China. *Science of the Total Environment,* 578**,** 523-534.

ZHANG, W., PARKER, K. M., LUO, Y., WAN, S., WALLACE, L. L. & HU, S. 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. *Global Change Biology,* 11**,** 266-277.

ZHANG, Y., HAN, X., HE, N., LONG, M., HUANG, J., ZHANG, G., WANG, Q. & HAN, X. 2014b. Increase in ammonia volatilization from soil in response to N

deposition in Inner Mongolia grasslands. *Atmospheric Environment,* 84**,** 156-162.

ZHAO, Z., DONG, S., JIANG, X., LIU, S., JI, H., LI, Y., HAN, Y. & SHA, W. 2017. Effects of warming and nitrogen deposition on CH4, CO2 and N2O emissions in alpine grassland ecosystems of the Qinghai-Tibetan Plateau. *Science of The Total Environment,* 592**,** 565-572.

ZHOU, L., ZHOU, X., ZHANG, B., LU, M., LUO, Y., LIU, L. & LI, B. 2014. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. *Glob Chang Biol,* 20**,** 2332-43.

ZHOU, X., WAN, S. & LUO, Y. 2007. Source components and interannual variability of soil CO2 efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biology,* 13**,** 761-775.

ZHOU, Y., HAGEDORN, F., ZHOU, C., JIANG, X., WANG, X. & LI, M.-H. 2016. Experimental warming of a mountain tundra increases soil CO2 effluxes and enhances CH4 and N2O uptake at Changbai Mountain, China. 6**,** 21108.

ZHOU, Y. M., TANG, J. W., MELILLO, J. M., BUTLER, S. & MOHAN, J. E. 2011. Root standing crop and chemistry after six years of soil warming in a temperate forest. *Tree Physiology,* 31**,** 707-717.

ZHU, C., MA, Y., WU, H., SUN, T., LA PIERRE, K. J., SUN, Z. & YU, Q. 2016. Divergent Effects of Nitrogen Addition on Soil Respiration in a Semiarid Grassland. *Scientific Reports,* 6**,** 33541.

ZHU, X., LUO, C., WANG, S., ZHANG, Z., CUI, S., BAO, X., JIANG, L., LI, Y., LI, X., WANG, Q. & ZHOU, Y. 2015. Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau. *Agricultural and Forest Meteorology,* 214**,** 506-514.

ZHUANG, Q., CHEN, M., XU, K., TANG, J., SAIKAWA, E., LU, Y., MELILLO, J. M., PRINN, R. G. & MCGUIRE, A. D. 2013. Response of global soil consumption of atmospheric methane to changes in atmospheric climate and nitrogen deposition. *Global Biogeochemical Cycles,* 27**,** 650-663.

ZONG, N., SHI, P. L., CHAI, X., JIANG, J., ZHANG, X. Z. & SONG, M. H. 2017. Responses of ecosystem respiration to nitrogen enrichment and clipping mediated by soil acidification in an alpine meadow. *Pedobiologia,* 60**,** 1-10.

ZUUR, A. F., IENO, E. N., WALKER, N. J., SAVELIEV, A. A. & SMITH, G. M. 2011. *Mixed Effects Models and Extensions in Ecology with R,* Springer.

Appendix

Appendix 1

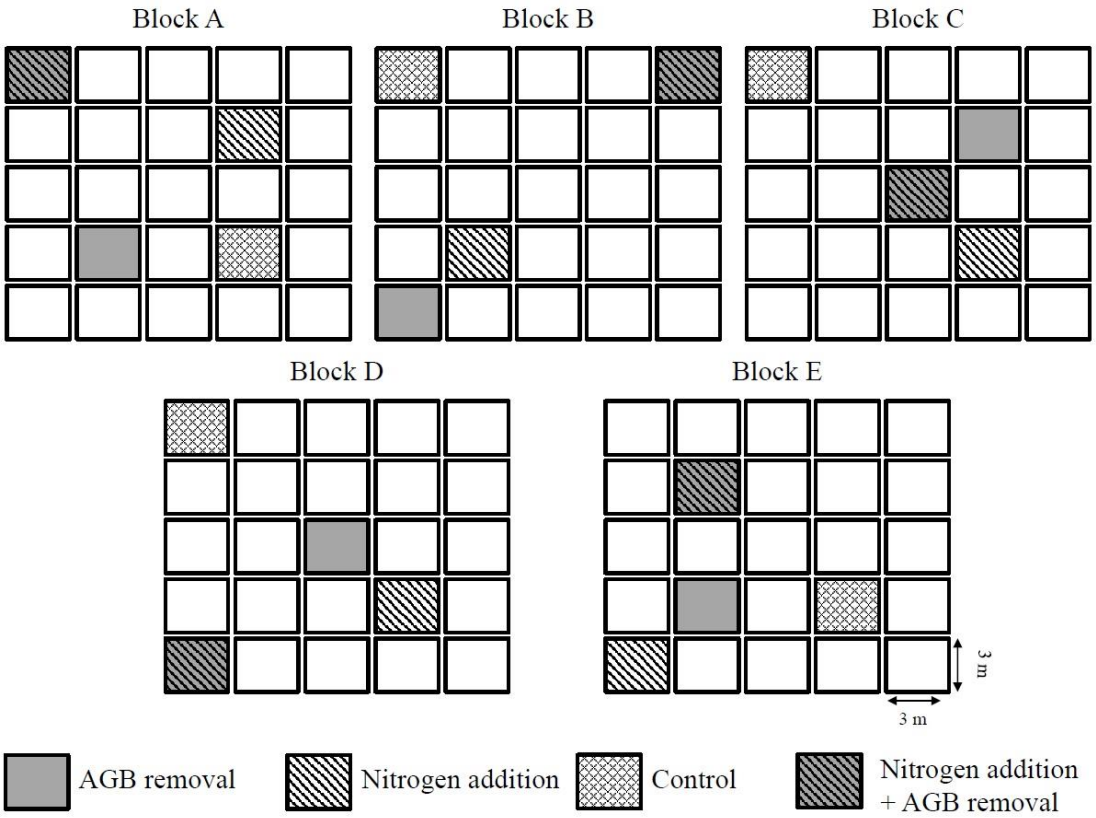

Figure A1.1 Randomised field experiment in a full-factorial design at Hazelrigg Field Station, Lancaster University, UK. Treatments consisted of: soil-only control, warming-only, nitrogen addition-only, above-ground (AGB) removal-only and the interactions AGB removal + warming, nitrogen + warming, AGB removal + nitrogen, AGB removal + warming + nitrogen, with five replicates (one within each block). There is a warming chamber in each of the selected plots (20 warming chambers in total).

Figure A1.2 Experimental area at Hazelrigg Weather Station, Lancaster, UK comprising of twenty-five plots in each of the five blocks (125 plots in total).

Figure A1.3 Open-top passive conical chamber to test warming effect, based on International Tundra Experiment design (ITEX: Marion et al., 1997).

Appendix 2

Figure A2.1 In-growth cores used to partition soil below-ground components: a) 1 µm mesh; b) 35 µm mesh and c) 2 mm mesh installed in the main treatments design (Chapter 2).

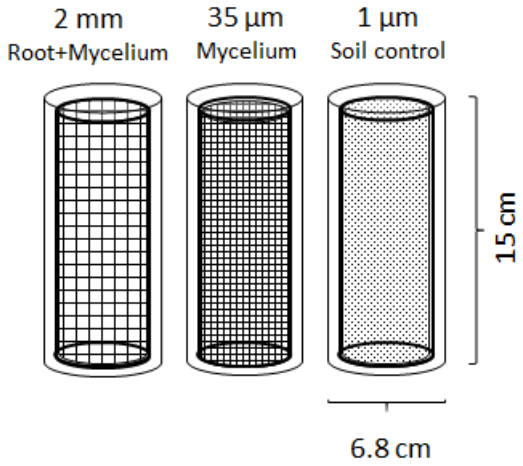

Figure A2.2 In-growth core design used to partition below-ground components. Root/mycelia cores allowed root and mycelial in-growth using a 2 mm mesh; mycelia cores excluded roots but allowed mycelial in-growth (35 µm mesh) and no in-growth was achieved through using 1 µm mesh.

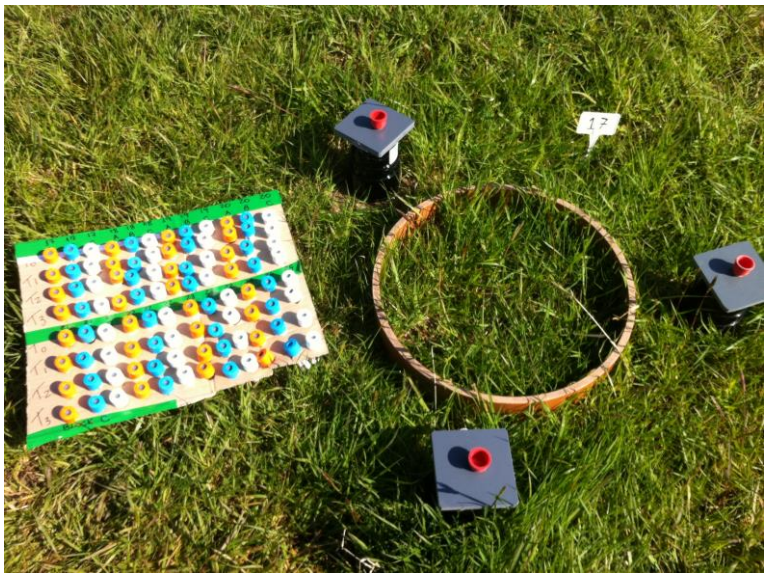

Figure A2.3 Greenhouse gas chamber lids were made using drainage pipe fitted with a lid containing a septum for gas sampling of each in-growth core over May-June 2016.

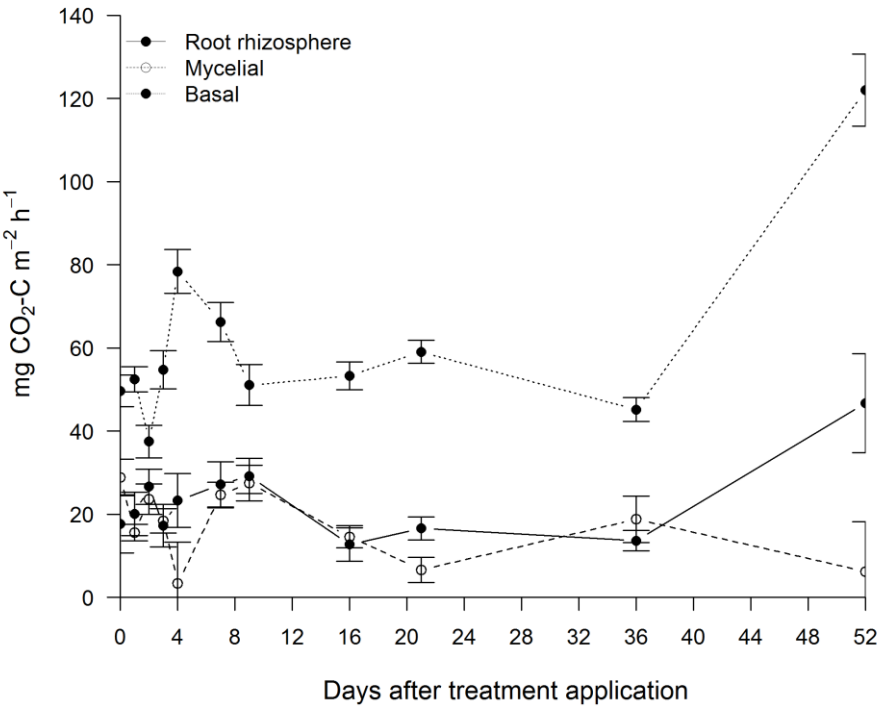

Figure A2.4 Root rhizosphere, mycelial and basal respiration over the growing season. Data are mean of all treatments and repetitions (n=40).

Table A2.1 Effects warming (warm), AGB removal, nitrogen addition (nadd) and partitioned below-ground (PBG) on ecosystem respiration. Significance tests using likelihood ratio test (LRT) comparing models with or without parameter of interest where degree of freedom (d.f.) shows the difference in degrees of freedom between the models. Significant effect (P<0.05) are shown in bold.

	CO_2 emissions mg CO_2-C m^{-2} h^{-1}		
	d.f.	LRT	P value
Warm	1	2.64	0.10
AGB removal	1	2.66	0.10
Nadd	1	3.19	0.07
PBG	1	72.28	**<0.0001**
Warm *x* AGB removal	1	0.48	0.49
Warm X PBG	1	0.34	0.56
Warm *x* Nadd	1	1.37	0.24
Nadd x PBG	1	0.01	0.83
AGB removal x PBG	1	0.54	0.46
AGB removal *x* Nadd	1	0.46	0.50
Warm *x* Nadd *x* AGB removal	1	0.01	0.93
Warm *x* Nadd *x* PBG	1	0.13	0.72
Warm *x* PBG *x* AGB removal	1	0.03	0.86
PBG *x* Nadd *x* AGB removal	1	0.01	0.92
Warm *x* Nadd *x* AGB removal x PBG	1	1.73	0.19

Appendix 3

Figure A3.1 A controlled temperature mesocosm experiment to quantify the interactive effect legume proportion and nitrogen addition, in a grass-legume mixture. One legume and one grass species grown in mixtures in different proportions (0, 25%, 50%, 75% and 100% legume abundance) were superimposed with N addition, giving a total of 10 treatments (50 pots).

Figure A3.2 Details of mesocosms pots and click seal lids for GHG measurements in the controlled temperature mesocosm experiment.

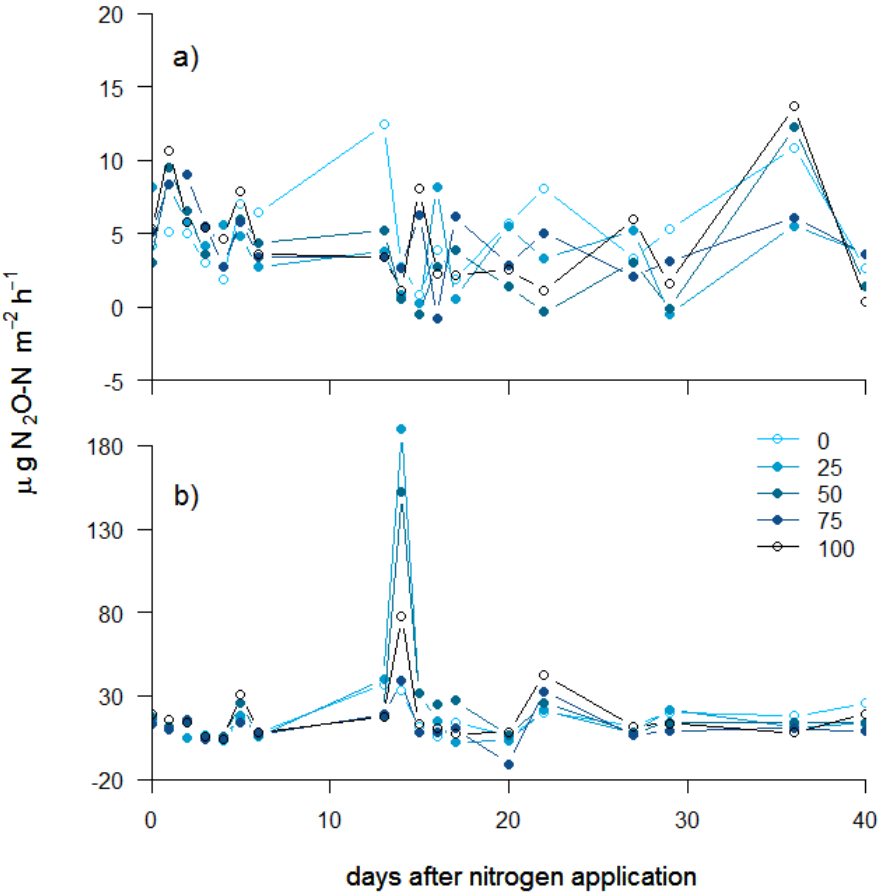

Figure A3.3 N_2O emission from the soil in response to legume proportion and nitrogen addition in the controlled temperature mesocosm experiment; a) without nitrogen and, b) with nitrogen. Data are mean (n=5).

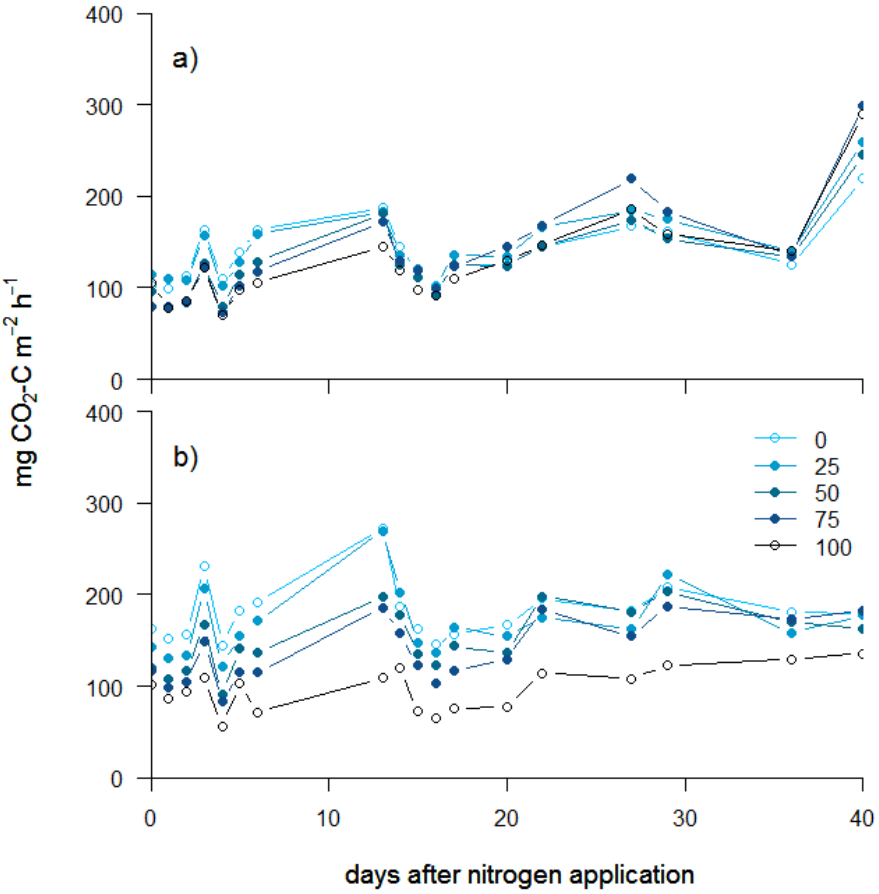

Figure A3.4 Ecosystem respiration in response to legume proportion and nitrogen addition in the controlled temperature mesocosm experiment: a) without nitrogen and, b) with nitrogen. Data are mean (n=5).

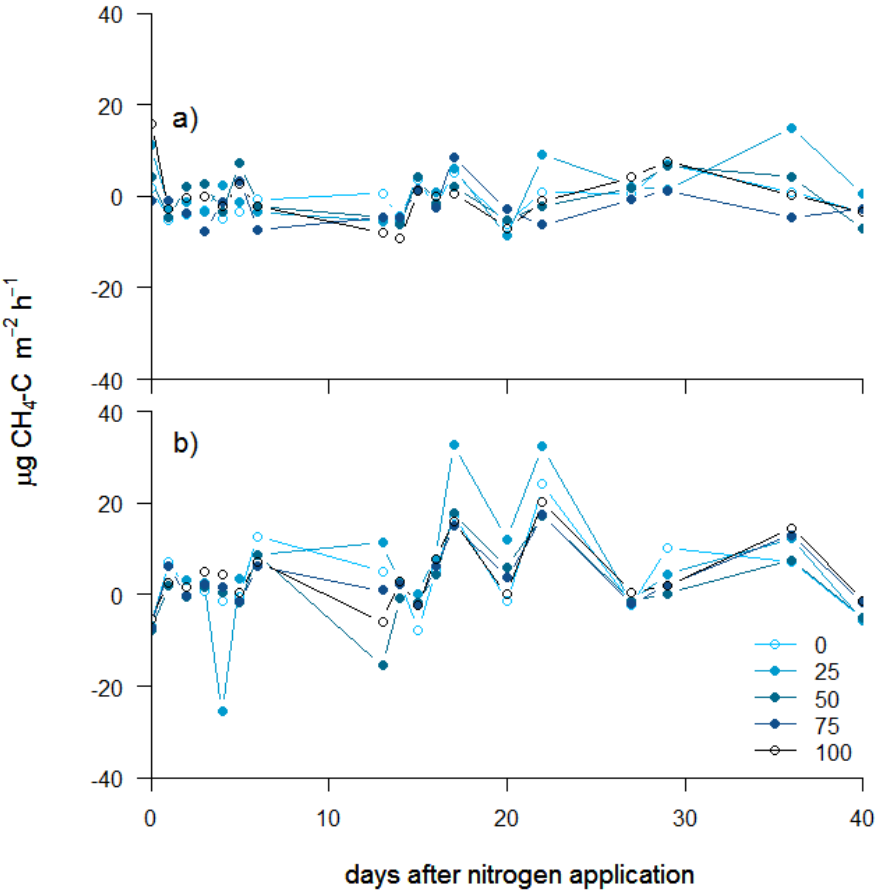

Figure A3.5 CH_4 emission from the soil in response to legume proportion and nitrogen addition in the controlled temperature mesocosm experiment; a) without nitrogen and, b) with nitrogen. Data are mean (n=5).

Table A3.1 Effects of legume biomass (LEG_BIOM) and nitrogen addition (NADD) on the cumulative emissions of N_2O, CO_2, CH_4. Significant effects ($P < 0.05$) are shown in bold.

		N_2O emissions mg N_2O-N m^{-2}		CO_2 emissions g CO_2-C m^{-2}		CH_4 emissions mg CH_4-C m^{-2}	
	d.f.	F value	*P*	F value	*P*	F value	*P*
LEG linear (LEG_BIOM)	1	3.73	0.06	6.2	**0.017**	2.65	0.11
NADD	1	59.07	**<0.0001**	0.4	0.51	32.25	**<0.0001**
LEG quadratic (LEG_BIOM^2)	1	1.09	0.30	2.1	0.15	0.97	0.33
LEG cubic (LEG_BIOM^3)	1	0.33	0.57	3.4	0.07	5.81	**0.02**
LEG_BIOM x NADD	1	0.05	0.82	15.6	**0.0003**	0.02	0.89
LEG_BIOM^2 x NADD	1	0.13	0.72	3.4	0.071	1.78	0.19
LEG_BIOM^3 x NADD	1	0.94	0.34	0.3	0.58	0.32	0.57

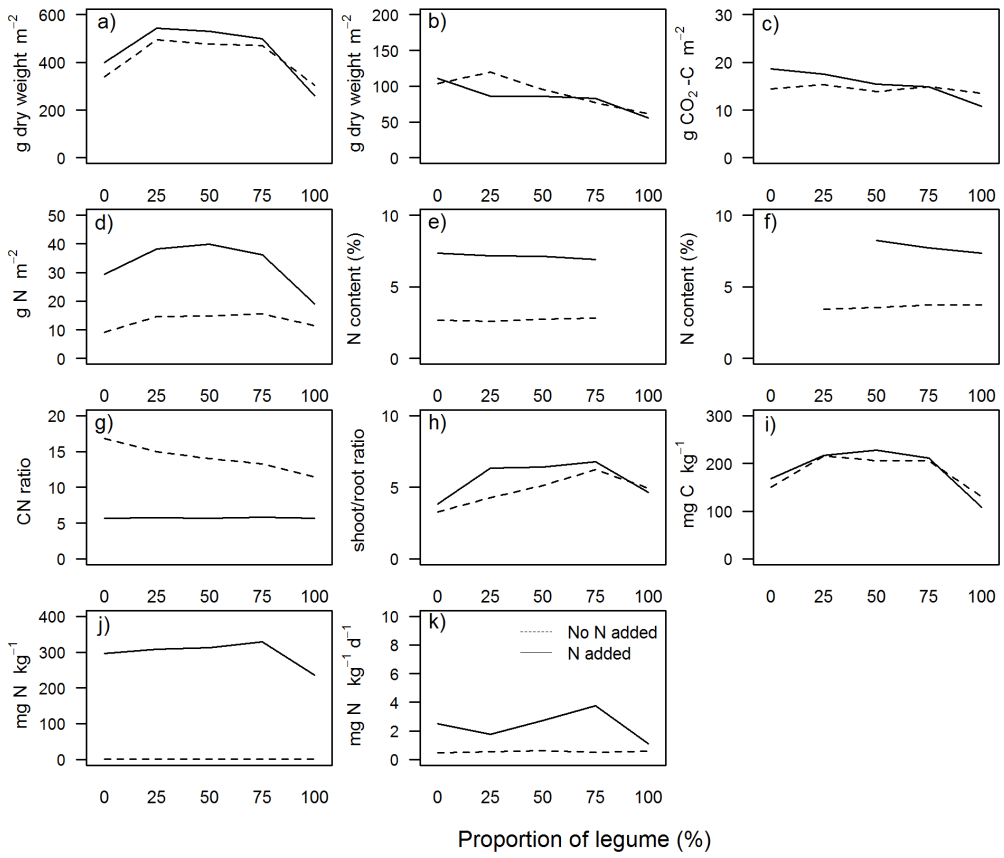

Figure A3.6 Interactive effect of legume proportion and nitrogen addition on a) above-ground biomass, b) below-ground biomass, c) ecosystem respiration, d) shoot N uptake, e) N (%) in grass, f) N (%) in legumes, g) shoot CN ratio, h) shoot/root ratio, i) total soil C, j) soil NH_4^+-N and k) net mineralisation rate.

Appendix 4

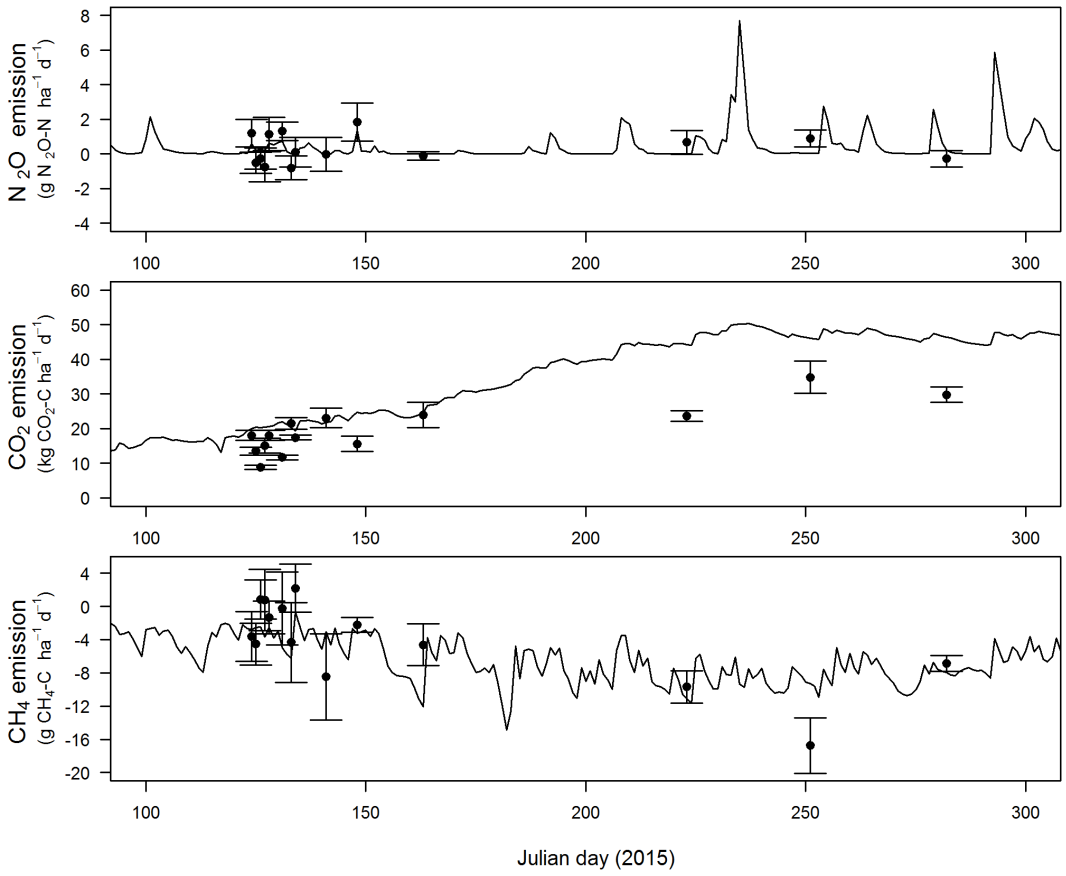

Figure A4.1 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the control treatments from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

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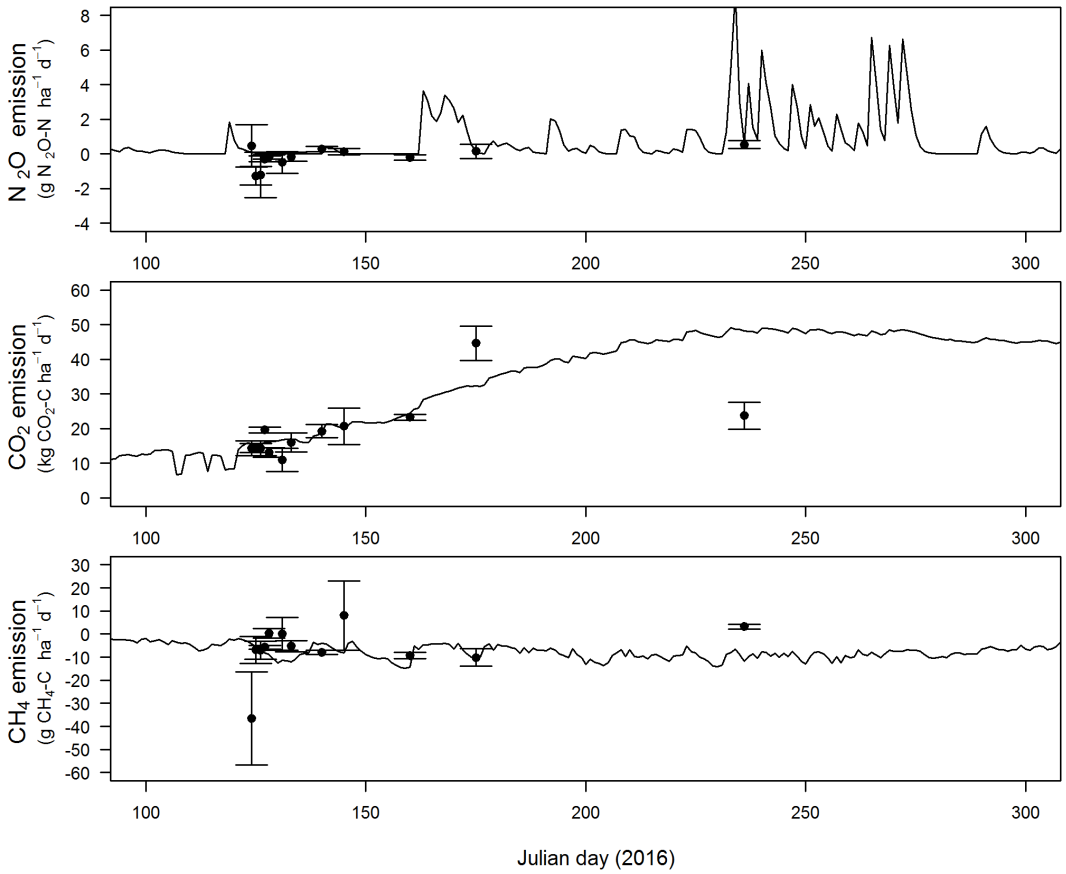

Figure A4.2 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the control treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

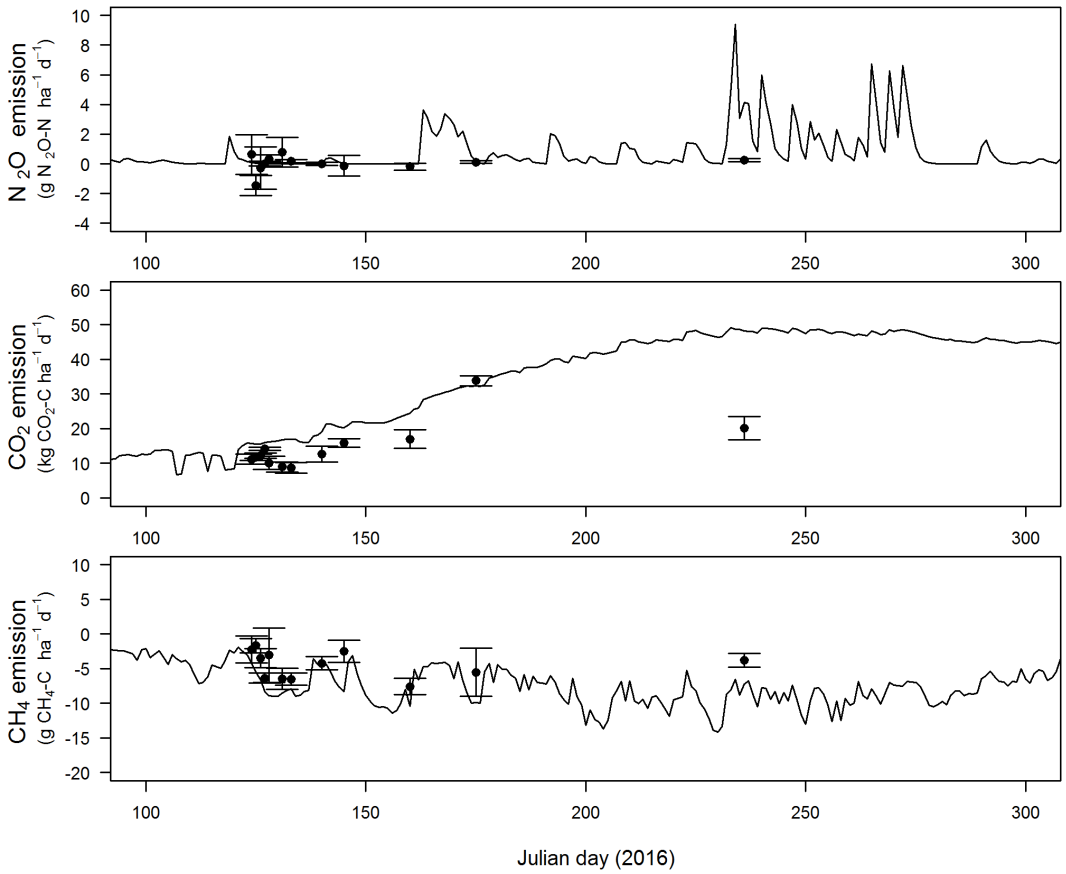

Figure A4.3 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the cutting treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

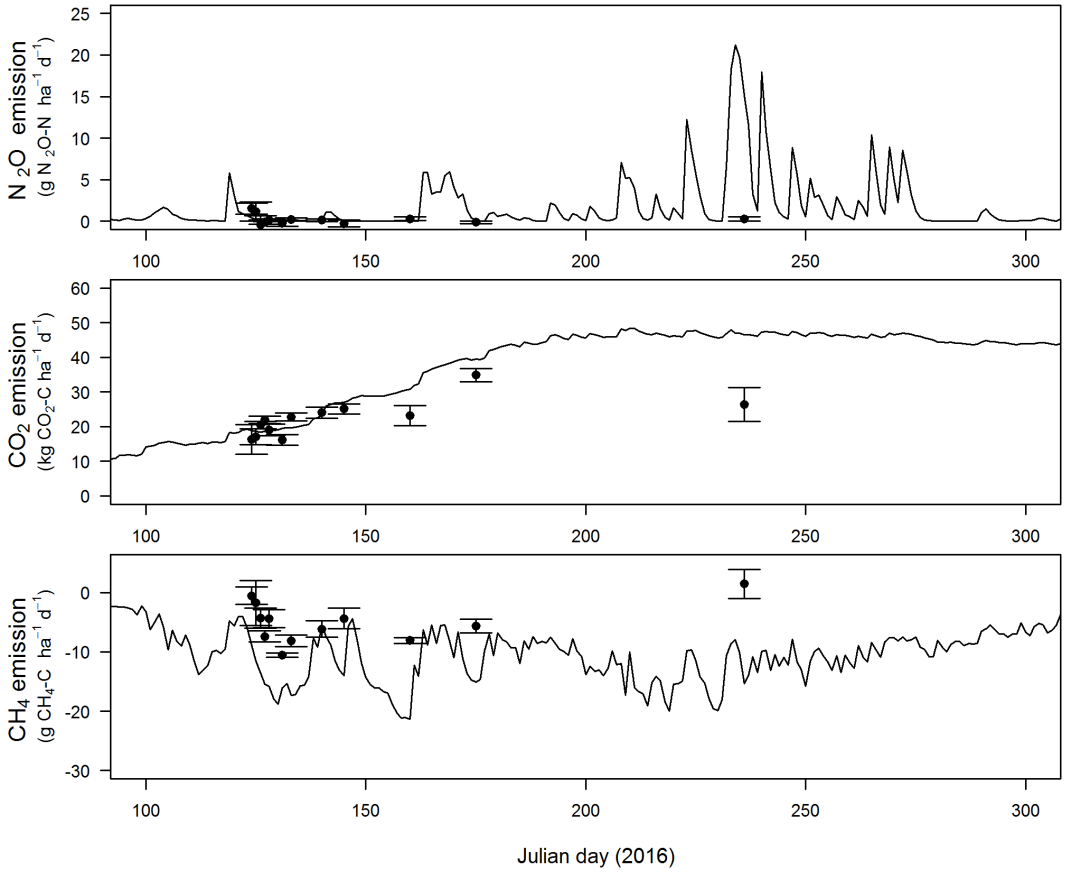

Figure A4.4 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the warming treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

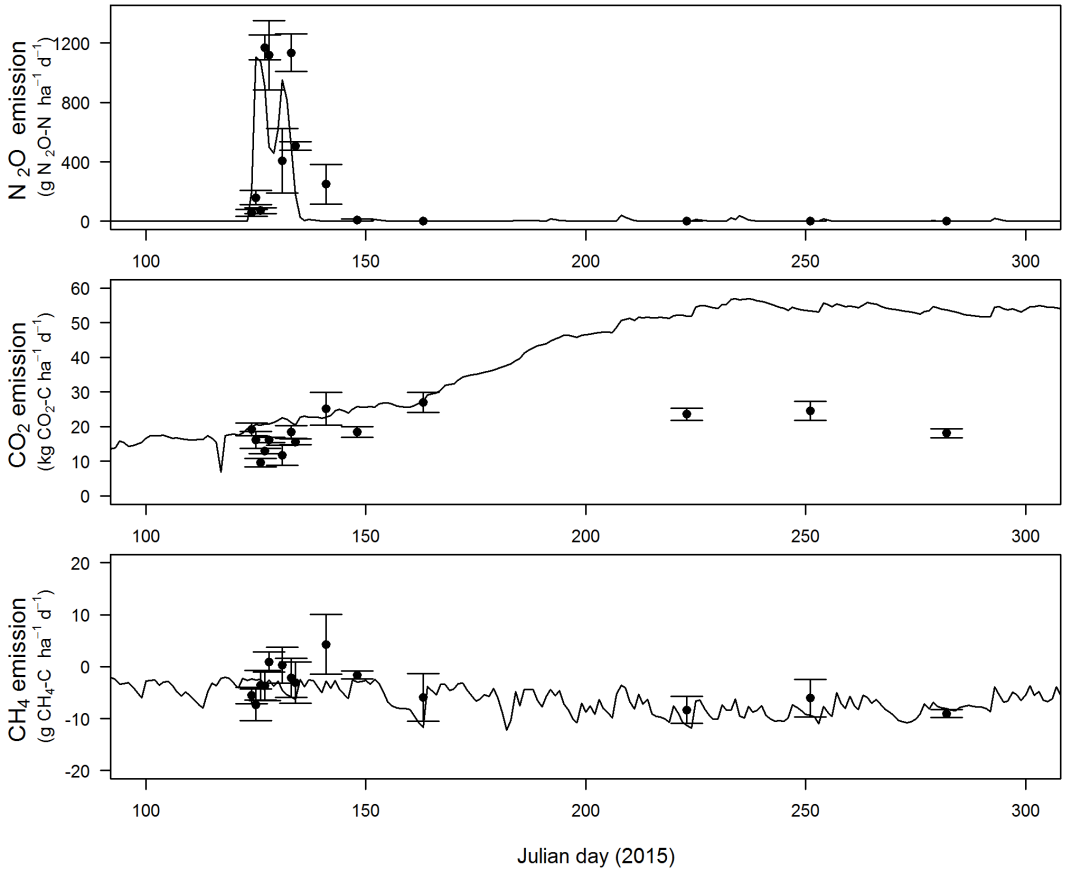

Figure A4.5 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between nitrogen and cutting treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

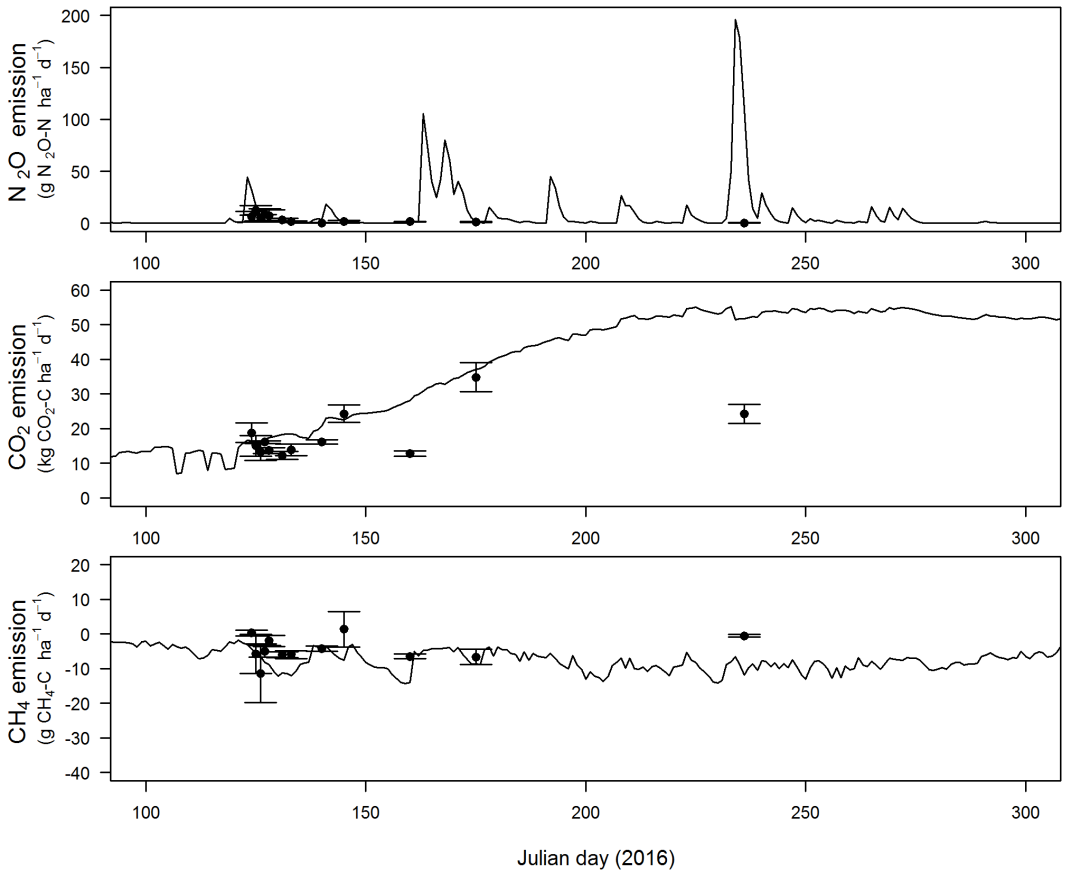

Figure A4.6 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between nitrogen and cutting treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

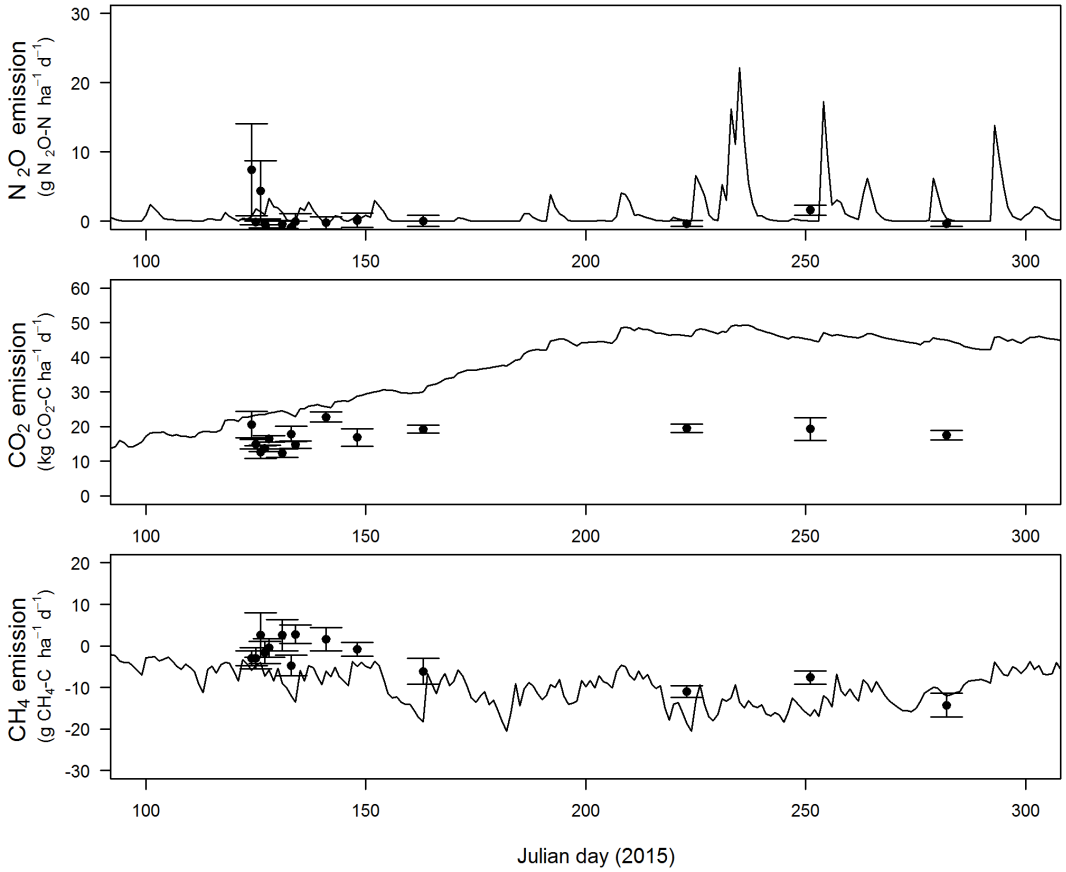

Figure A4.7 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between warming and cutting treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

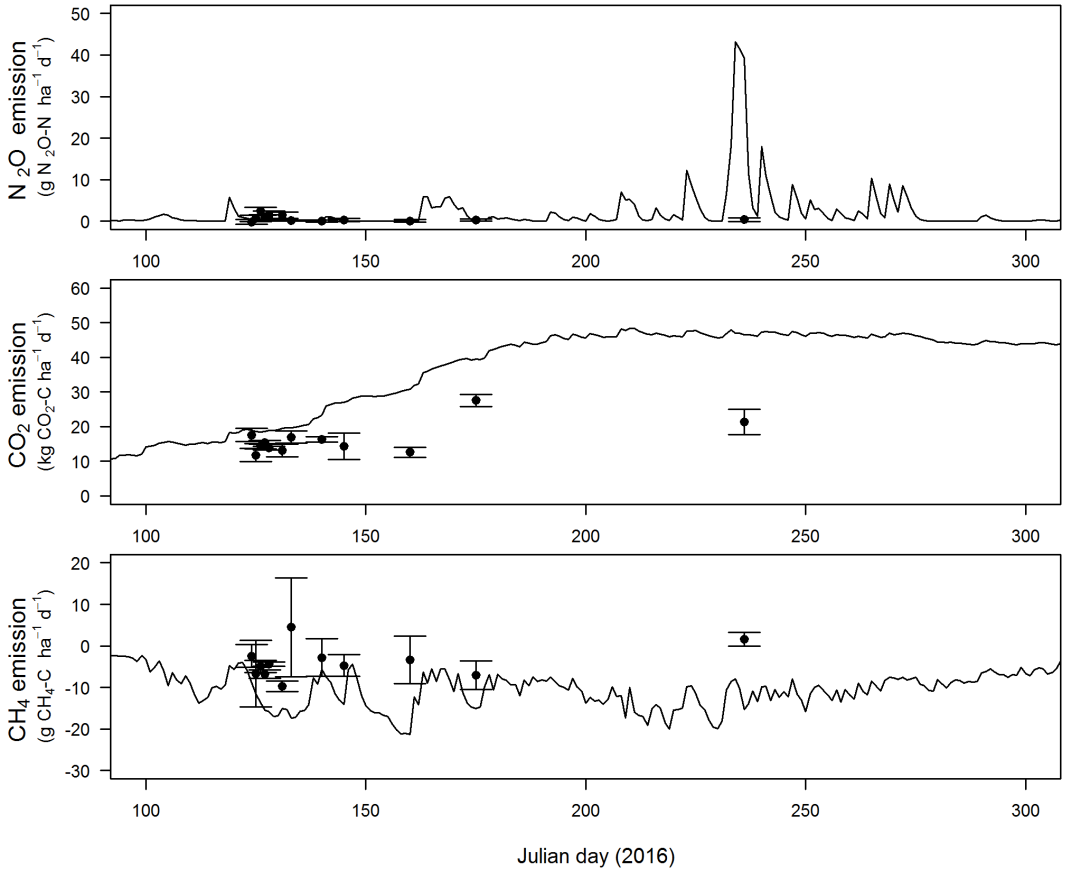

Figure A4.8 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between warming and cutting treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.

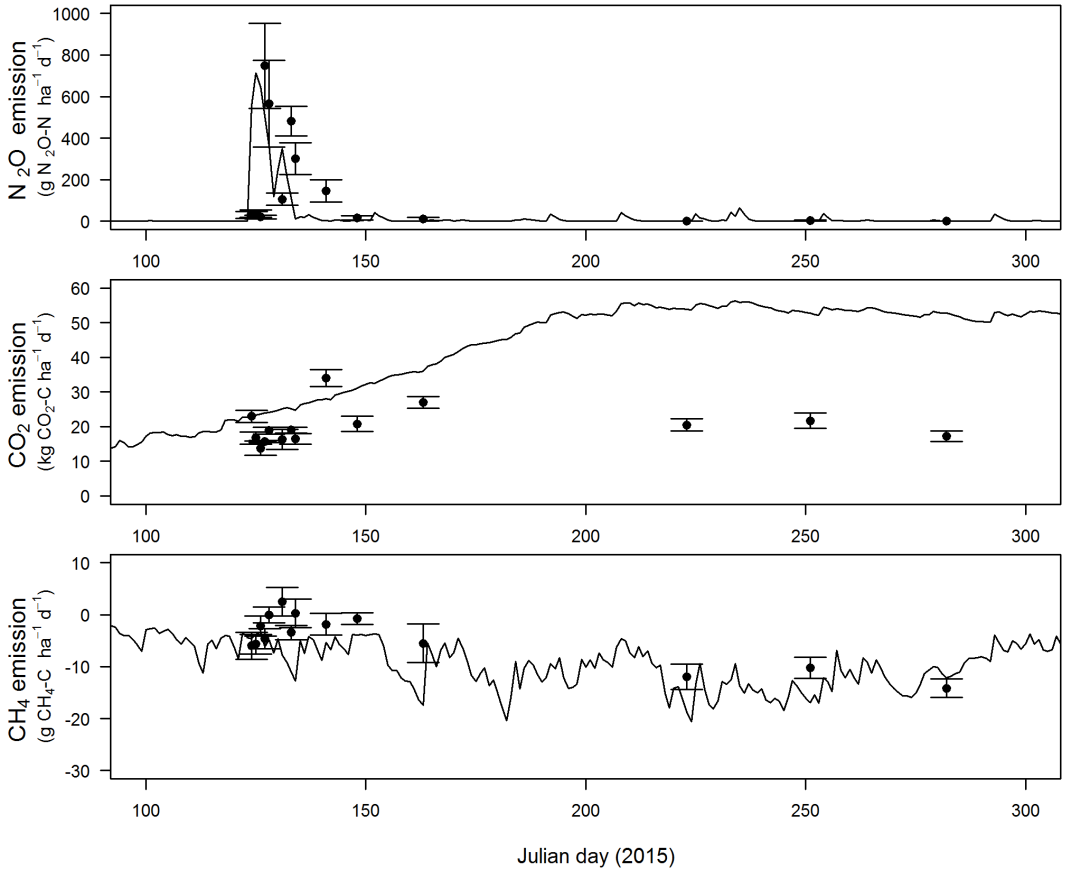

Figure A4.9 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between warming, cutting and nitrogen treatment from the growing season of 2015. Error bars are standard deviations for 5 repetitions.

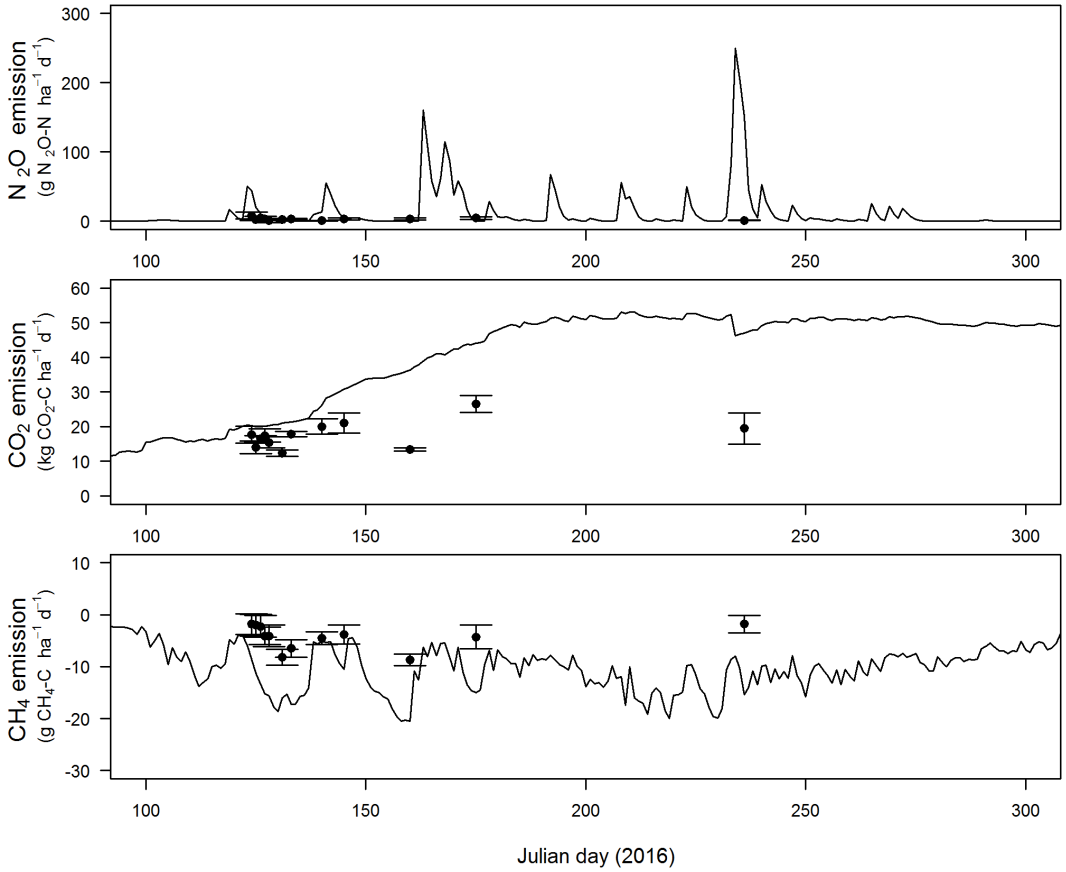

Figure A4.10 Measured (filled circle) and simulated (solid line) N_2O, CO_2 and CH_4 emissions from soils of the interaction between warming, cutting and nitrogen treatment from the growing season of 2016. Error bars are standard deviations for 5 repetitions.