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Impacts of land use change to short rotation
forestry for bioenergy on soil greenhouse gas
emissions and soil carbon

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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 are acknowledged.

Name:

Date: 02/09/2015

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Abstract

Short Rotation Forestry (SRF) for bioenergy could be used to meet biomass requirements and contribute to achieving renewable energy targets. As an important source of biomass it is important to gain an understanding of the implications of large-scale application of SRF on the soil-atmosphere greenhouse gas (GHG) exchange. This study examined the effects of land use change (LUC) from grassland to SRF on soil fluxes of methane (CH₄), nitrous oxide (N₂O) and carbon dioxide (CO₂), and the important drivers in action.

Examining soils from a range of sites across the UK, CO₂ emission potentials were reduced under SRF with differences between coniferous and broadleaved transitions; these changes were found to be related to changes in soil pH and microbial biomass. However, there were limited effects of SRF tree species type on CH₄ and N₂O fluxes. A detailed study at an experimental SRF site over 16 months demonstrated a reduction in CH₄ and net CO₂ emissions from soils under SRF and revealed intriguing temporal dynamics of N₂O under Sitka spruce and common alder. A significant proportion of the variation in soil N₂O fluxes was attributed to differences between tree species, water table depth, spatial effects, and their interactions. The effects of microtopography (ridges, troughs, flats), and its interactions with water table depth on soil GHG fluxes under different tree species was tested using mesocosm cores collected in the field. Microtopography did not significantly affect soil GHG fluxes but trends suggested that considering this spatial factor in sampling regimes could be important. N₂O fluxes from Sitka spruce soils did not respond to water table depth manipulation in the laboratory suggesting that they may also be determined by tree-driven nitrogen (N) availability, with other research showing N deposition to be higher in coniferous plantations. An N addition experiment led to increased N₂O emissions with greatest relative response in the Sitka spruce soils.

Overall, LUC from rough grassland to SRF resulted in a reduction in soil CH₄ emissions, increased N₂O emissions and a reduction or no change in net CO₂ emissions. These changes in emissions were influenced both directly and indirectly

by tree species type with Sitka spruce having the greatest effect on N₂O in particular, thus highlighting the importance of considering soil N₂O emissions in any life cycle analysis or GHG budgets of LUC to SRF for bioenergy. This research can help inform decisions around SRF tree species selection in future large-scale bioenergy planting.

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Abbreviations

BD – bulk density

C – carbon

CH₄ – methane

CO₂ – carbon dioxide

CO_{2eq.} – carbon dioxide equivalent

ECD – electron capture detector

FID – flame ionisation detector

GC – gas chromatograph

GHG – greenhouse gas

GMC – gravimetric moisture content

GWP – global warming potential

N – nitrogen

N₂O – nitrous oxide

NH₄⁺ – ammonium

NO₃⁻ – nitrate

PPM – parts per million

PPB – parts per billion

SOC – soil organic carbon

SOL – soil organic layer

SOM – soil organic matter

SRC – short rotation coppice

SRF – short rotation forestry

WHC – water holding capacity

WFPS – water-filled pore space

Chapter 1. Introduction and research aims

The global rise in temperature over the last century has been driven by increasing concentrations of atmospheric greenhouse gases (GHGs) such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), among others. Anthropogenic activities such as fossil fuel combustion and land use change (LUC) have been the main contributors to these rising GHG concentrations (IPCC, 2007). Global warming is set to continue through the 21st century, with predicted increases in temperature of more than 2 °C combined with a more frequent occurrence of extreme weather events, which will greatly affect ecosystem functioning if urgent action is not taken (IPCC, 2014). Climate stabilisation can be best achieved by reducing GHG emissions through the replacement of fossil fuels with renewable energy sources and by enhancing sequestration by maximising uptake of GHGs in the biosphere (Schulze et al., 2009; Ter-Mikaelian et al., 2015).

Bioenergy is part of a suite of renewable energy sources under consideration as an alternative to fossil fuels with potential for GHG mitigation (Field et al., 2007; Don et al., 2012; Timilsina & Shrestha, 2011). Short rotation forestry (SRF) is one of the bioenergy crops currently being considered due to its high biomass yield capability, and its potential for GHG uptake and carbon (C) storage (Mckay, 2011). However, knowledge is currently limited regarding its large-scale potential and sustainability (Harris et al., 2015). This study focuses on the effects of LUC from grassland to various SRF species on soil GHG emissions and soil C.

1.1 Bioenergy and policy

Bioenergy is the production and use of energy or fuels from biomass feedstocks on a renewable basis. It is one of the most versatile forms of low C and renewable energy, with the potential to contribute to the generation of energy for electricity, heat and transport (Don et al., 2011; DECC, 2012). Bioenergy includes first and second generation biofuels for transport (bioethanol and biodiesel), gaseous fuels (e.g. Methane (CH₄) captured from landfills) as an alternative to natural gas, and solid

biomass fuels such as firewood and biomass pellets for heating and electricity generation (Field et al., 2007; Whitaker et al., 2010; Don et al., 2011). First-generation bioenergy is produced from food crops, while second-generation bioenergy is derived from cellulosic, typically woody materials (Bartle & Abadi, 2010). Bioenergy provides approximately 10 % of the global renewable energy supply and accounts for about 80 % of the total renewable energy contribution (IEA, 2011). Some estimates suggest that 15-25 % of the world's global energy demands by 2050 could be met through biomass production (Beringer et al., 2011), but this depends on land availability and sustainable yields (Smith et al., 2010). The European Union has committed to increase the proportion of renewable energy from 9% in 2010 to 20% of total energy consumption by 2020 (EU, 2009) and currently bioenergy contributes approximately two-thirds of total renewable energy in Europe (IEA, 2011). As set out in the 2011 UK Renewable Energy Roadmap, bioenergy is an important part of the Government's plans to meet the Renewable Energy Directive objectives in 2020 (target of 15% renewable energy by 2020). Bioenergy also has a role to play if the UK is to meet its low carbon objectives by 2050, which is a reduction in GHG emissions by at least 80% below 1990 levels (DECC, 2011). Only 3% of primary energy in the UK, however, is currently produced from bioenergy feedstocks (DECC, 2012). This is expected to increase in response to growing pressures for the decarbonisation of energy supply and as a result of technical advances in the use of lignocellulosic biomass for the production of liquid or gaseous fuels as well as for combustion for heat and power (Chum et al., 2012).

The cost effectiveness of bioenergy (biomass) compared with other renewable energy technologies (Timmons et al., 2015) makes it an attractive option for contributing towards the delivery of renewable energy targets. Despite this, there are a number of concerns associated with biomass production and its sustainability: its C and GHG reduction potential, regarding land availability of bioenergy production and the consequences for food production, and the potential environmental impacts on air quality, biodiversity and water resources (Cowie et al., 2006; Rowe et al., 2009;

Whitaker et al., 2010; Don et al., 2011; Osborne & Jones, 2012). The original rationale for supporting bioenergy relied largely on the assumption that it could deliver genuine GHG emissions savings based on the principal that there is a negative balance between the C emitted during combustion and that fixed during photosynthesis. This was an oversimplification and it is now recognised that the production of biomass energy is not C neutral due to GHG emissions released during establishment and ground preparation, crop growth, land management, harvesting, processing and transportation (Field et al., 2007; Searchinger et al., 2009; Don et al., 2011; Haberl et al., 2012). In addition, there may also be significant consequences of land use change (LUC) to bioenergy crops on soil C and GHG emissions (Fargione et al., 2008; Searchinger et al., 2008; Keith et al., 2015; Parmar et al., 2015). These previously neglected GHG emissions could potentially offset any C savings via reduced fossil fuel use and could increase the risk of bioenergy crops becoming C positive (Don et al., 2011; Zenone et al., 2015). Despite this, research and modelling work to date, albeit limited, suggests that bioenergy has the potential to mitigate GHG emissions and increase soil C storage (Hastings et al., 2009; Rowe et al., 2009; Dondini et al., 2014; Keith et al., 2015). However, in a meta-analysis carried out by Harris et al. (2015) it was identified that this potential for C saving and GHG mitigation is dependent on the original land use and the bioenergy crop being planted.

1.2 Bioenergy and land use change

Land use change (LUC) is second only to fossil fuel combustion as a source of global GHG emissions (IPCC, 2007). Djomo and Ceulemans (2012) define LUC as “changes in the areal extent of a particular land use over a given time period and within a given spatial entity”. From 1850-1998 approximately 270 Gt of C was emitted to the atmosphere as CO₂ from fossil fuel combustion, and approximately 136 Gt as a result of LUC, with most of this coming from deforestation (Watson et al., 2000).

Land use patterns have changed through time as a result of human needs, affected by advances in technology, government objectives and targets, environmental issues, and economic change (Rounsevell & Reay, 2009). Now, in order to help meet

international and European renewable energy and GHG emissions reduction targets, a significant amount of land may be converted to bioenergy production (DECC, 2011, Zona et al., 2013; Harris et al., 2015). The demand for land for biomass energy production augments the existing demands of agriculture and forestry which are already under pressure due to rising demands for food, goods and recreation from a growing population (Timilsina & Shrestha, 2011). Competition for land between energy crops and food should be minimal with second generation bioenergy crops, since they are generally established on abandoned agricultural land or marginal land (Dondini et al., 2014). In most cases soil C in this land is already substantially depleted, compared to its starting state, (>30%) as a result of cultivation or erosion (Grigal & Berguson, 1998). Recent estimates indicated that 35,000 km² of land could potentially be made available for perennial energy crops in the UK without impacting on high quality agricultural land used for food production (Lovett et al., 2014). If this was achieved then up to 66% of the UK's heat and 62% of electricity demands could be met (Wang et al., 2014a). Therefore, if considerable LUC takes place as a consequence of wide-scale bioenergy production, it is important to quantify the direct effects that this will have on the GHG balance and soil C stocks.

Changes in land use greatly affect the cycling and storage of C and soil-atmosphere GHG exchange in ecosystems (Guo and Gifford, 2002; Rounsevell & Reay, 2009; Neumann-Cosel et al., 2011), and the magnitude of change in C storage depends on how physical, chemical and biological processes are altered over time under different land uses and management scenarios (Watson et al., 2000). Over recent years there has been an on-going debate about the C and GHG balance associated with bioenergy crop production and the direct and indirect effects of LUC (Harris et al., 2015). The direct effects are dependent on the transition type (i.e. the original land use and the type of bioenergy crop planned), due to differences in initial C stocks, crop related inputs and nutrient turnover, fertiliser requirement, ground preparation, management and harvesting regime, amongst others. For example, a recent meta-analysis of 138 studies by Harris et al. (2015) found that the transition from arable

land use to short rotation coppice (SRC) or perennial grasses resulted in an increase in soil organic carbon (SOC), transition from grassland to SRC left SOC unchanged, and transitions from grassland to perennial grasses and forest to SRC both resulted in a reduction in SOC. Harris et al. (2015) found insufficient data to carry out a full meta-analysis for GHGs and highlighted the significant knowledge gap existing for the effects of LUC to bioenergy on soil-atmosphere GHG exchange. A further multi-site study by Keith et al. (2015), examining the effects of LUC from agriculture (mostly grasslands) to different SRF species types on soil C, found that planting coniferous SRF species resulted in increased C stock compared to broadleaved species, which had no effect, while *Eucalyptus* species reduced soil C. Keith et al. (2015) also flag the need for bioenergy LUC effects on soil GHGs to be quantified, so that changes in soil C can be considered together with changes in soil-atmosphere GHG exchange in order to better predict the impacts of different transitions overall. It is also important to consider initial soil C stocks and crop rotation length when attempting to quantify LUC impacts. Land uses with high initial SOC such as grasslands on organic soils could be more susceptible to the effects of LUC to bioenergy crops (Poeplau et al., 2011) as conversion could deplete soil C quite rapidly (Don et al., 2011) while it can take years to recover (Poeplau et al., 2011).

Based on existing research, in the UK there are a number of potential LUC transitions scenarios to first and second generation bioenergy crops from grassland, arable and forestry land uses, and these are summarised in Table 1. Some transitions are more likely than others; for example, LUC from forestry to non-forest bioenergy crops is unlikely, and transitions to first generation crops are being increasingly challenged on sustainability grounds (Hill, 2007; Gomez et al., 2008). It has been suggested that first generation crops are unlikely to have a role in UK bioenergy supply post 2020 due to developments in the production of second generation bioenergy crops for transport fuels (Heaton et al., 2008; Rowe et al., 2009). Hence, it is important to focus current and future research on transitions that are most likely such as from grassland or arable land to SRF, where greater uncertainties lie due to their novelty.

A number of non-bioenergy related meta-analyses on LUC and soil C have been published in the last decade on particular land use transitions, for example, from forest to agricultural land use (Murty et al., 2002), afforestation of agricultural land (Paul et al., 2002; Laganière et al., 2010), and across a range of transitions (Guo and Gifford, 2002). The meta-analysis by Guo and Gifford (2002) looked at the effects of LUC on soil C using 74 primary studies from across 16 countries. Increases in soil C were shown for LUC from forest to pasture (+8%), crop to pasture (+19%), crop to plantation forest (+18%) and crop to secondary forest (+53%), while decreases were shown for pasture to crop (-59%), forest to crop (-42%), forest to plantation (-13%), and pasture to plantation (-10%) (Guo & Gifford, 2002). These findings highlight the importance of previous land use on observed changes in soil C, with the greatest potential for soil C gains deriving from changing from crops to forestry (Laganière et al., 2010). Furthermore, tree species type appears to have an effect on soil C content; Guo and Gifford (2002) found that broadleaf plantations had little effect on soil C change while conifers such as pine reduced soil C by up to 12%. A later study by Laganière et al. (2010) found broadleaved plantations to have a positive effect on soil C following transition from agricultural land to forestry compared to pine and *Eucalyptus* species. Therefore, it is not only important to consider the original land use and the transitional land use in general terms, but also to consider the effects that LUC to and from individual species may have on soil C and GHG emissions.

Table 1.1 Summary table of possible bioenergy land use transition in the UK, the likelihood of certain transitions both currently and in the future and our knowledge of the possible effects on soil properties. Likelihood refers to the likelihood of a given transition to bioenergy happening now. Arrows under future likelihood simply indicate direction of change, not the magnitude of direction (Anderson-Teixeira et al., 2006; Dawson and Smith 2007; Gomez et al., 2008; Guo and Gifford, 2002; Heaton et al. 2008; Hill 2007; Post and Kwon 2000; RFA, 2008; Rowe et al., 2009).

Bioenergy crop		Original land use								
		Grass			Arable			Forest		
		Likelihood	Future Likelihood	Knowledge	Likelihood	Future Likelihood	Knowledge	Likelihood	Future Likelihood	Knowledge
1 st gen.	Sugar Beet	Med	↓	Good	V. High	↓	Good	Low	↓	Good
	Wheat	Med	↓	Good	V. High	↓	Good	Low	↓	Good
	Oil Seed Rape	Med	↓	Good	V. High	↓	Good	Low	↓	Good
2 nd gen.	Miscanthus	Med	↑	Poor	High	↑	Poor	Low	↑	Poor
	SRC	Med	↑	Poor	High	↑	Poor	Low	↑	Poor
	SRF	Med	↑	Poor	High	↑	Poor	Low	↑	Poor

The effects of LUC to bioenergy on soil C and GHG exchange may be dependent on the environmental context (Hastings et al., 2009; Hillier et al., 2009). Laganière et al. (2010) showed that climatic zone had a significant effect on change in SOC following afforestation of agricultural land, with the greatest increase found under a temperate maritime climate, such as that in the UK. However, climatic differences at a smaller regional scale could impact bioenergy crop yields and the soil environment (Aylott et al., 2010). Soil type could also modify the impact of LUC to bioenergy on soil C and GHG emissions. Laganière et al. (2010) showed that there is a greater increase in SOC following afforestation of agricultural land in soils with a high clay content and with a high pH. Paul et al. (2002) also noted the clay content of soil can influence changes in soil C following afforestation. Identifying scenarios (e.g., particular combinations of climate, soil type and crop) where changes in soil C are positive and soil GHG emissions are reduced is of great importance.

1.3 Soil-atmosphere greenhouse gas exchange

1.3.1 Soil greenhouse gases

Greenhouse gases exist naturally in the atmosphere absorbing and emitting radiation within the thermal infrared range, and without them the earth would be too cold for human habitation (IPCC, 2007). However, since pre-industrial times atmospheric concentrations of the three primary GHGs (CO_2 , CH_4 and N_2O) have risen dramatically from 280 ppm to 391 ppm, from 715 ppb to 1803 ppb and from 270 ppb to 324 ppb in 2011, respectively (IPCC, 2013). This increase has been attributed to anthropogenic activities such as fossil fuel burning, land use change and intensified agriculture, resulting in the net effect of global warming through increased radiative forcing (IPCC, 2014). These GHGs are long lived in the atmosphere and have assigned global warming potentials (GWP's) based on their radiative forcing, mean lifetime and emissions. Although, CH_4 and N_2O are termed trace gases, due to their relative low concentrations compared to CO_2 , their GWP on a molar mass basis over 100 years are 298 and 34 times greater than a unit of CO_2 , respectively (IPCC, 2013). Soils are

important sources and sinks for these three major radiative forcing GHGs (Smith et al., 2007).

1.3.1.1 Soil carbon and respiration

Globally soils contain approximately 2500 Gt C (Lal, 2004), almost as much C as that of the atmosphere and terrestrial vegetation combined (Schimel, 1995). The distribution of this C varies with depth in the soil profile, with higher concentrations in the top one metre (Batjes, 1996). The amount of C in any soil depends on the type of ecosystem, the land use (current and historical) and the management scenario (Jobbágy and Jackson 2000). Carbon accumulation in soil represents the long-term net balance of photosynthesis and total respiration in terrestrial ecosystems (Schlesinger, 1990).

CO₂ is removed from the atmosphere by plants during photosynthesis and approximately half is assimilated into the plant biomass where it is then allocated to leaves, stems, roots, branches and seeds (Raich & Schlesinger, 1992; Morison et al., 2012). The remainder is released again to the atmosphere via a variety of processes termed collectively as ecosystem respiration (Trumbore, 2006). Ecosystem respiration is a product of both autotrophic respiration (i.e. that derived from living plant leaves stems and roots), and heterotrophic respiration which is as a result of the decomposition of non-living SOM and soil surface litter by soil organisms (Trumbore, 2006). The difference between photosynthesis and ecosystem respiration is termed net ecosystem exchange (NEE) (Luo & Zhou, 2006). Soil respiration is the second largest global C flux after photosynthesis, estimated annually at around 80 Gt C (Raich & Potter, 1995) which is almost 10 times the annual amount of C emitted by burning fossil fuels (Marland et al., 2008), making it a key component of the global C balance (Raich et al., 2002). Heterotrophic respiration is most responsive to variables that control microbial activity such as temperature, soil moisture, nutrient availability and the quantity and quality of substrate available for decomposition (Hartley & Ineson, 2008; Bradford et al., 2010; Strickland et al., 2010). Whereas, autotrophic respiration is predominantly driven by photosynthetic rates (Tang et al., 2005;

Gomez-Casanovas et al., 2012) with some influence of temperature, the quantity of biomass, nutrient content and the supply of sugars for photosynthesis (Ryan et al., 1997).

1.3.1.2 Soil methane (CH₄) fluxes

CH₄ is produced in soils mainly by methanogenic bacteria during the decomposition of organic material under anaerobic conditions and accounts for more than a third of all CH₄ emissions (Smith & Conen, 2004; McNamara et al., 2008). In upland soils CH₄ can also be produced inside soil aggregates where anaerobic microsites occur (Dutaur and Verchot, 2007). Net CH₄ emissions are commonly associated with poorly drained organic soils (McNamara et al., 2008), peatlands (Frolking et al., 2011) and rice paddy fields (Neue and Sass, 1994). Soils can also act as a sink for CH₄, through the oxidation (uptake) of CH₄ in the soil by methanotrophic bacteria under aerobic conditions (Hanson & Hanson, 1996). Uptake rates are generally low in agricultural soils due to the disturbance from agricultural practices and the addition of nitrogen (N) fertiliser which is known to inhibit CH₄ oxidation (Stuedler et al., 1989; Hütsch, 2001; Smith et al., 2000). Soil CH₄ emissions to the atmosphere are calculated from the net balance between production and oxidation (Chan and Parkin, 2001). However, in many soils methanogenic and methanotrophic bacteria co-exist making it difficult to determine if certain soils are net sources or net sinks for CH₄ (McNamara et al., 2008). CH₄ emissions are affected by a range of environmental and edaphic factors that regulate the activities of methanogenic and methanotrophic bacteria and the exchange of gases (McNamara et al., 2008). Important factors include water table depth (Moore & Dalva, 1993; Ball et al., 1997; Frenzel & Karofeld, 2000; Mojeremane et al., 2010), soil moisture (Castro et al., 1994a; Gundersen et al., 2012), temperature (Crill et al., 1994; Christiansen & Gundersen, 2011), and soil diffusion (Ball et al., 1997; Dong et al., 1998). The influence of these factors on the direction and magnitude of CH₄ fluxes will vary depending on soil type, habitat, and plant species.

1.3.1.3 Soil nitrous oxide (N₂O) fluxes

Soils are a major source of atmospheric N₂O emissions which occur from both natural and agricultural sources (IPCC, 2007; Wang et al., 2014b). Although emissions are highest from agricultural soils (Mosier et al., 1998), mainly due to the application of nitrogen rich fertilisers (Dobbie *et al.*, 1999; Skiba & Smith, 2000), it is now recognised that forest soils may also represent a significant source of N₂O (Zhang et al., 2008). N₂O in soils is mainly produced from the two contrasting microbial processes of nitrification (De Boer & Kowalchuk, 2001) and denitrification (Gillam et al., 2008), and overall contributes approximately 70% to global N₂O emissions (Syakila & Kroeze, 2011). Nitrification and denitrification can occur simultaneously in soils but the rate at which each is occurring will depend on soil abiotic conditions (Butterbach-bahl et al., 2013; Wang et al., 2014b). Nitrification is an aerobic process and involves oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) and then to nitrate (NO₃⁻). When the concentration of oxygen is limited nitrifying bacteria can use NO₂⁻ and reduce it to NO and N₂O (Smith *et al.*, 2003). Denitrification is an anaerobic process and involves the reduction of NO₃⁻ to N₂O (and N₂). The largest emissions of N₂O are generally linked to denitrification but conditions for nitrification are more common so these fluxes are not trivial (Skiba & Smith, 2000). N₂O can also be consumed by soil microbes if environmental conditions are suitable, however, this process is still under debate (Chapuis-Lardy et al., 2007; Schlesinger, 2013). A number of abiotic and biotic factors control N₂O fluxes in soils. Depth to water table (Martikainen et al., 1993; Huttunen et al., 2003; Ball et al., 2007; Zenone et al., 2015) and soil water content (which determines water filled pore space (WFPS)) (Davidson, 1991; Dobbie *et al.*, 1999; Davidson et al., 2000; Christiansen & Gundersen, 2011; Gundersen et al., 2012; Butterbach-bahl et al., 2013) are important. Research has shown that N₂O emissions are optimum at 70–80% (WFPS) depending on soil type (Davidson et al., 2000). Other important factors include, Soil N availability (Ryden & Lund, 1980; Liu & Greaver, 2009; Gundersen et al., 2012; Zenone et al., 2015), depth of the organic (O) horizon (Borken & Brumme, 1997; Borken & Beese, 2006), soil pH (Weislien et al., 2009;

Gundersen et al., 2012), and temperature (Keeney et al., 1979; Skiba & Smith, 2000; Smith *et al.*, 2003; Borken & Beese, 2006; Butterbach-bahl et al., 2013). N₂O fluxes and the abiotic and biotic factors controlling them will be discussed in the context of SRF in more detail below.

1.3.2 Role of soil microbes in the GHG and C balance

Soil microbes are central to the functioning of terrestrial ecosystems, and as mentioned above they play important roles in carbon and nitrogen cycling, SOM decomposition, and soil-atmosphere GHG exchange (Zak et al., 2003; Waldrop & Firestone, 2006). The soil biological community is extremely diverse with up to 50 000 bacterial species and 200 m of fungal hyphae existing in just 1 g of soil (Bardgett et al., 1993). Soil bacteria and fungi both decompose organic matter via the production of extracellular enzymes and the abundance of each in any soil is greatly determined by the chemical composition of the litter inputs (Waldrop & Firestone, 2004; Bray et al., 2012). Generally, microbial communities in soils that have poor quality litter inputs tend to have higher fungal:bacterial biomass than those with high quality litter inputs (Paustian & Schnürer, 1987; Gallo et al., 2004; Waldrop & Firestone, 2004). Soil bacteria can be further divided in to Gram-negative and Gram-positive bacterial groups. Gram-negative tend to dominate in soils with higher available organic matter and nitrogen availability (Fierer et al., 2003; Potthoff et al., 2006; Hossain et al., 2010), whereas Gram-positive bacteria and fungi are more abundant in soils with lower quality litter inputs (Bray et al., 2012). Microbes respond to changes in C dynamics driven by changes in the plant community (Kampichler et al., 1998; Kowalchuk et al., 2002), therefore, LUC to bioenergy could result in shifts in microbial community composition which could affect soil-atmosphere GHG exchange.

1.4. Short Rotation Forestry for bioenergy

1.4.1 What is Short Rotation Forestry?

SRF is the practice of growing high density plantations (>2500 trees ha⁻¹) of fast-growing native and non-native tree species on short rotational lengths (>10 years) and harvesting when the diameter at breast height (DBH) is 10–20 cm (Hardcastle, 2006; McKay, 2011; Leslie et al., 2012). This silvicultural system is particularly suitable for bioenergy crop applications as it provides relatively high yields over short time frames (Proe et al., 2002; Hardcastle et al., 2006; Hoffmann & Weih, 2005; McKay, 2011). SRF could provide added flexibility to the woody bioenergy supply in the UK as, unlike coppice crops, harvesting can take place year round, and the product has a lower bark and moisture content and a higher density making it an ideal fuel source (Hardcastle et al., 2006; Leslie et al., 2012). Biomass yields of SRF may also be higher than coppice systems per unit area (McKay, 2011). There are a number of coniferous and broadleaved species currently being considered as potential biomass sources (Hardcastle, 2006; McKay, 2011; Leslie et al., 2012; Keith et al., 2015). However, experience of SRF in Britain is currently limited, although the Forestry Commission have been carrying out DECC funded trials at seven sites across England using various species including *Eucalyptus* species. *Eucalypts* are also grown successfully in warmer countries for biomass production such as in north-western Spain, where conditions are favourable for the *globulus* variety of this non-native species (González-García et al., 2009).

1.4.2 Current understanding of SRF effects on soil C and GHG emissions

As a result of its novelty, current knowledge and datasets on SRF effects on soil C and soil GHG emissions are limited. A review carried out by McKay (2011) on the growth and environmental impacts of SRF suggested that LUC from arable crops to SRF could result in significant increases in soil C but that the effect of LUC from grassland was uncertain. McKay (2011) also suggested that any C gain would be tree species dependent due to differences in the quality and quantity of inputs between

broadleaves and coniferous species and their differential effects on the soil environment in general. A recent meta-analysis of 138 studies by Harris et al. (2015) examined the effects of LUC to second generation bioenergy crops on soil C and GHG and reported no change in soil C following transition from grassland to short rotation coppice systems. However, they were unable to quantify transitions from grassland to SRF as a result of insufficient available data and highlighted this as an area of research importance.

Subsequently, the first detailed UK study and dataset on LUC effects of planting SRF for bioenergy on soil C stocks was carried out by Keith et al. (2015). This study examined soils following LUC from agricultural systems (mainly grasslands) to SRF collected from 11 different sites across the UK. It was observed that planting coniferous species led to an increase in soil C compared to the original land use as a result of high litter accumulation. While planting broadleaved species, although results were highly variable, resulted in no change in soil C stocks compared to the original land use. This work contributed to filling the knowledge gap surrounding the sustainability of SRF as a bioenergy feedstock, but no measurements of soil-atmosphere GHG exchange were made. In order to determine the true GHG mitigation potential of an energy crop changes in GHG fluxes need to be quantified (Smith et al., 2013).

Although there is a lack of data on SRF and soil GHG emissions, knowledge can be drawn on from what is known about forestry in general. A review carried out by Dalal and Allen (2008) quantified GHG fluxes from natural ecosystems at a global scale and found that temperate forests have the highest CH₄ uptake rates of all natural systems. Dalal and Allen (2008) also recognised that contrary to prior belief, temperate forest soils could also be significant sources of N₂O emissions. The strength of a forest soil's sink or source potential will be affected by soil environment, climatic characteristics, tree species and forest growth stage (Barrena et al., 2013). In general terms CH₄ uptake rates are larger in forest systems as a result of physical and biogeochemical changes in the soil environment that favour methanotrophic bacteria.

For example, porosity in forest soils is usually larger than in agricultural soils as a result of increased SOM and reduced compaction and disturbance, which increases the soil diffusion potential of the soil which aids CH₄ oxidation (Ball et al., 1997; Prieme et al., 1997; Christiansen & Gundersen, 2011). Water table drawdown as a result of high demand for water by trees can create aerobic conditions in the soil that are optimal for methanotrophic activity (Hanson & Hanson, 1996). Certain tree species may enhance soil CH₄ uptake more than others as a result of their differential effects on the soil (Borken & Beese, 2006; Yavitt & Williams, 2015). For example, coniferous trees are known to reduce soil pH which can in-turn regulate the activity of soil methanotrophs which are sensitive to soil acidity (Amaral et al., 1998). Borken et al. (2003) reported higher CH₄ uptake in beech forest soils compared to Scots pine whilst other studies have found no difference in soil CH₄ fluxes between tree species on similar soils (McNamara et al., 2008; Christiansen & Gundersen 2011). CH₄ uptake suppression in response to high levels of available N can also occur in forest systems, where N-fixing species are planted or in areas of high atmospheric N deposition (Butterbach-bahl et al., 1998; Reay et al., 2001; Reay & Nedwell, 2004).

Dalal & Allen (2008) have estimated that temperate forests have the potential to emit up to 8.07 kg N₂O-N ha⁻¹ y⁻¹ which is by no means insignificant considering the high GWP of this species. Soil N₂O production via the microbial processes of nitrification and denitrification is largely regulated by the availability of inorganic N (NH₄⁺ and NO₃⁻) (Liu & Greaver, 2009). This inorganic N can be delivered to the soil system via atmospheric deposition, via litter inputs or by fixation. This is of particular importance in forests where N deposition is known to be very high and in tree species selection, for example common alder fixes atmospheric N via actinomyorrhizal nodules and can therefore have high levels of NO₃⁻ in the soil and its litter (Reay et al., 2005). It is also well documented that deposition of atmospheric inorganic N is larger in coniferous forests than in deciduous (Rothe et al., 2002; Gundersen et al., 2009). Therefore, coniferous stands receiving more N than adjacent broadleaved stands could potentially release more N₂O as a result of increased N availability in

the soil. It has recently been discovered that soil fungi can directly contribute to soil N₂O production, however, its significance is still under debate (Prendergast-Miller et al., 2011; Chen et al., 2014; Maeda et al., 2015). Prendergast-Miller et al. (2011) demonstrated under laboratory conditions that ectomycorrhizal fungi extracted from Sitka spruce root tips could produce N₂O from nitrate production. As soil fungi are particularly dominant in acidic forest soils and are tolerant of high inorganic N concentrations (Chen et al., 2014) their contribution to N₂O emissions may not be insignificant.

1.5 Research aims and experimental approach

The urgent need to reduce global GHG emissions through the use of alternative energy sources to fossil fuels has been highlighted in this review. SRF is a promising biomass source that could contribute to such mitigation, and as a crop it is well suited to temperate climates such as that in the UK. Although forests soils are generally significant C sinks and have the potential to offset anthropogenic atmospheric GHG increases, the magnitude and dynamics of LUC effects could be impacted by the original land use, tree species, soil type and climatic conditions.

The recent multi-site study by Keith et al. (2015) found that LUC from grassland to coniferous species resulted in an increase in soil C stock but did not take soil GHG emissions into consideration. The consideration of GHGs (CO₂, CH₄ and N₂O) could have an impact on the overall sustainability of LUC and in some cases there is a risk of soil GHG emissions offsetting the CO₂ uptake by the plantation (Zenone et al., 2015).

The overall aim of this research was to investigate the effects of LUC from grassland to various SRF species on soil emissions of the primary greenhouse gases (CO₂, CH₄ and N₂O), and to examine the importance of key factors and potential mechanisms underlying these effects. To address this aim, a combination of laboratory (Chapter 2), field (Chapter 3) and mesocosm (Chapter 4) studies were undertaken.

Chapter 2 first investigated GHG flux potentials in short term laboratory incubations from soils under grassland and a variety of tree species across six different sites in the UK. Differences between tree species type were examined and the effects of LUC on CO₂ efflux were then related to changes in soil C stock and soil microbial community composition. This work was integrated as part of a UK wide campaign being undertaken for the Ecosystem Land Use Modelling consortium project led by the Centre for Ecology & Hydrology in Lancaster. This broad approach was followed by more focused in-depth approaches utilising a single experimental site.

Having identified broad LUC effects across a range of sites, Chapter 3 went on to investigate soil GHG emissions under grassland and monocultures of three different tree species over a 16 month period at the Gisburn Forest Experimental site (this site was examined in Chapter 2). The relative importance of a range of soil physical and chemical variables for GHG fluxes were assessed.

Chapter 4 describes a medium-term manipulation study and an additional N addition experiment using intact mesocosms collected from the field site used in Chapter 3. This work extended the investigation of tree species effects on soil GHG emissions to focus on how water table depth and microtopography (as created during planting) may modify tree species effects on GHG emissions.

Chapter 2: Bioenergy driven land use change impacts on soil greenhouse gas regulation under Short Rotation Forestry

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Declaration of contribution

Sample collection for this chapter was carried out as part of the larger ELUM project (detailed in research aims section of the general introduction) by Kim Parmar, Dr Aidan Keith, Dr Rebecca Rowe and Danny Tregidgo. The laboratory incubation experiment was conducted solely by Kim Parmar whilst PLFA analysis were carried out by Dr Claudia Moeckel and Dr Gloria Pereira. Data were analysed with help from Dr Aidan Keith and the publication was written by Kim Parmar and reviewed by Dr Aidan Keith, Dr Niall McNamara and Dr Saran Sohi.

2.1 Abstract

Second-generation bioenergy crops, including Short Rotation Forestry (SRF), have the potential to contribute to greenhouse gas (GHG) emissions savings through reduced soil GHG fluxes and greater soil C sequestration. If we are to predict the magnitude of any such GHG benefits a better understanding is needed of the effect of land use change (LUC) on the underlying factors which regulate GHG fluxes. Under controlled conditions we measured soil GHG flux potentials, and associated soil physico-chemical and microbial community characteristics for a range of LUC transitions from grassland land uses to SRF. These involved ten broadleaved and seven coniferous transitions. Differences in GHGs and microbial community composition assessed by phospholipid fatty acids (PLFA) profiles were detected between land uses, with distinctions between broadleaved and coniferous tree species. Compared to grassland controls, CO₂ flux, total PLFAs and fungal PLFAs (expressed per g C), were lower under coniferous species but unaffected under broadleaved tree species. There were no significant differences in N₂O and CH₄ flux rates between grassland, broadleaved and coniferous land uses, though both CH₄ and N₂O tended to have greater uptake under broadleaved species in the upper soil layer. Effect sizes of changes across LUC transitions of CO₂ flux and, soil pH, total PLFA and fungal PLFA were positively related. These relationships between fluxes and microbial community suggest that LUC to SRF may drive change in soil respiration by altering the composition of the soil microbial community. These findings support that LUC to SRF for bioenergy can contribute towards C savings and GHG mitigation.

2.2 Introduction

Bioenergy currently accounts for almost two-thirds of the total renewable energy in Europe and much of this comes from energy crops (Osborne & Jones, 2012). Furthermore, the European Union has committed to increase the proportion of renewable energy from 9% in 2010 to 20% of total energy consumption by 2020 (EU, 2009). Although there are competing land demands from activities such as food production, infrastructure, recreation and biodiversity (Smith et al., 2013), the rationale remains for converting certain land to bioenergy crop production (POST, 2012). For a bioenergy crop to be considered as a viable and sustainable option in the future it must provide GHG savings in comparison to the use of fossil fuels (Rowe et al., 2009; Don et al., 2012). Impacts of LUC on GHG emission reduction are dependent on the land uses involved, but LUC to bioenergy has the potential to deliver GHG emissions savings through soil C sequestration, with the greatest potential following LUC from arable crops to forestry (Guo & Gifford, 2002; Laganière et al., 2010). In addition, and linked to changes in soil C, LUC can also influence GHG fluxes between the soil and the atmosphere (Houghton, 2003).

Short Rotation Forestry (SRF) could contribute to biomass requirements for renewable energy targets (Mckay, 2011; Leslie et al., 2012). Although not currently widely practised in the UK commercially, a suite of species is under consideration for SRF, including coniferous and broadleaved species types (Hardcastle et al., 2006; Mckay, 2011; Leslie et al., 2012). Tree species can influence soil organic carbon (SOC) sequestration and GHG fluxes due to varying rates of rhizodeposition (Paterson et al., 2007), differences in above and below-ground C partitioning (Mokany et al., 2006) and differences in litter inputs and decomposition rates (Vesterdal et al., 2008).

Litter decomposition rates are generally distinct between coniferous and broadleaved species, with litter decomposition most rapid for broadleaved species (Wedderburn & Carter, 1999; Peterken, 2001; Morison et al., 2012). Litter decomposition rates are strongly related to litter qualities including, litter N and lignin content, C/N ratio, and leaf area (Peterken, 2001; Reich et al., 2005; Hobbie et al., 2006; Vesterdal et al., 2008)

and these can vary greatly between tree species. Litter quality can also affect soil pH, which in turn can alter soil microbial activity affecting decomposition of soil organic matter (Morison et al., 2012). Roots also directly add organic material to the soil through exudation (rhizodeposition), fine root turnover and through coarse root shedding (Morison et al., 2012). Root-derived inputs (rhizodeposits) are chemically diverse and range in complexity from labile exudates to senescent material released as a consequence of tissue turnover (Paterson et al., 2009). These compounds provide a diverse source of substrate to soil microbial communities and are responsible for the stimulation of microbial biomass and activity in the rhizosphere (Paterson et al., 2009). Soil microbial community composition can be measured by analysis of phospholipid fatty acids (PLFAs). PLFA analysis has become widely used to study soil microbial communities (Zelles, 1997; Zelles, 1999) and quantifies total soil microbial biomass and the proportions of bacteria and fungi. Total PLFA is well-correlated with other methods for microbial biomass estimation and readily discriminate land use, soil type and land management practises (e.g. Bardgett et al., 1996).

Around half of soil respiration is derived from plant root respiration; the remaining respiration is associated with the decomposition of organic matter by the microbial community (Paterson et al., 2009; Morison et al., 2012). In the absence of root respiration, the rate of heterotrophic respiration (the CO₂ mainly derived from soil microbial activity) is largely a function of microbial community composition and organic matter quality, and ultimately organic matter quality is regulated by plant inputs (Wardle et al., 2004; Bardgett et al., 2008). Examining this component of respiration following LUC to SRF may give an indication of how changes in organic matter quality, or differences between species types, influence CO₂ fluxes. As emissions of methane (CH₄) and nitrous oxide (N₂O) contribute to climate change they must also be considered in LUC to forestry (Morison et al., 2012). It is generally accepted that forests are strong sinks for CH₄ (Smith et al., 2000). N₂O is a powerful GHG and has a global warming potential (GWP) 298 times that of CO₂ (IPCC, 2007).

Unlike CH₄ and CO₂, N₂O can be produced under both aerobic and anaerobic conditions and can be consumed in wet, nitrogen-poor soils (Chapuis-Lardy et al., 2007). Recent studies indicate a tendency towards higher N₂O emissions from deciduous than coniferous forest soils (Ambus et al., 2006; Pilegaard et al., 2006) due to differences in tree litter quality and soil moisture (Morison et al., 2012).

Previous work examining changes in soil C stock following the establishment of different SRF species has shown greater litter accumulation, and an overall increase in soil C stock in coniferous soils (relative to agricultural controls) compared to broadleaved soils (Keith et al., 2015). Despite broadleaved species having no overall effect on soil C stock, the response was more variable suggesting that individual species influence soil C accumulation differently. When combined with estimates of C stocks in aboveground biomass the likelihood of C accumulation under conifers was further strengthened (Keith et al., 2015). In addition to these findings on soil C, knowledge on GHG fluxes under SRF is needed to contribute to a better understanding of sustainability of this bioenergy land use. Therefore, this study examined potential soil GHG fluxes, under standardised conditions, from LUC transitions, and the associated changes in soil physico-chemical and soil microbial community characteristics. The gas flux measurements also yield additional information on the potential for the biological consumption and production of GHGs such as N₂O and CH₄. Specifically, this study tested for i) differences in GHG potential fluxes, soil physico-chemical (pH, % C) and microbial community characteristics between land uses (controls and different SRF species types), and ii) whether changes in soil physico-chemical (pH, % C) and microbial community characteristics could explain changes in CO₂ flux.

2.3 Materials and methods

2.3.1 Site selection and sampling strategy

Sampling was undertaken at six sites across the UK from replicated experimental and commercial SRF sites. A paired plots approach was used where SRF species and

adjacent land continuing in former land use could be identified at each location. To confirm that the soil for the control land use was comparable to the transitional SRF land use, data on management history and soil type had been collected and examined (Table. 1). Following soil sampling, texture analysis was carried out and was used to confirm similarity in soil type between control land use and transitional land use at each site (Table. 1). Expert advice and current literature on potential SRF tree species was also used to make an informed decision regarding suitable site selection (Proe et al., 2002; Hardcastle et al., 2006; McKay, 2011). The tree species chosen for this study, which have been broadly classified as coniferous (7 transitions) and broadleaved (10 transitions), included common alder (*Alnus glutinosa*), Ash (*Fraxinus excelsior*), Downy birch (*Betula pubescens*), Hybrid larch (*Larix x eurolepis*), Poplar (*Populus spp.*), Scots pine (*Pinus sylvestris*), Silver birch (*Betula pendula*), Sitka spruce (*Picea sitchensis*), and Sycamore (*Acer pseudoplatanus*). All sites with the exception of the site in North-West England (20 years into its second rotation; Table. 1) are in their first rotation ranging in age from 12 to 24 years.

A hierarchical sampling design was used to capture spatial variability (Keith et al., 2015). Five sampling locations were randomly selected within each paired plot (transition) (i.e. control or tree species) using an overlain grid. At each randomly selected sampling location, soil cores were taken from three positions, resulting in 15 spatially nested samples per transition.

Three soil cores (30 cm x 4.8 cm) were taken at each sampling location using a split-tube soil corer (Eijkelkamp Agrisearch Equipment BV, Giesbeek, The Netherlands), at the grid intersect and then at distances of 1 m and 1.5 m in random compass directions. Prior to soil sampling, the litter (L) and fermentation layers (L_f) were removed. Soil cores were divided into 0–15 cm and 15–30 cm sections in the field, bagged, and returned immediately to the laboratory where they were stored at 4 ° C.

Table 2.1 Details and soil characteristics of sampling locations used to examine the effects of Short Rotation Forestry on soil greenhouse gas regulation in GB. Land uses in bold represent control land use. Management terms; Pasture = grazed grassland, Rough Pasture = seasonally or un-grazed grassland, F = fertilised, NF = No Fertiliser applied. Soil type based on the Avery soil classification; texture class derived based on the Soil Survey of England & Wales texture classes. C stock values represent means \pm SD; n = 15. Table adapted from Keith et al. (2015).

Region	Lat	Long	Land use transition	Established	Management	Soil type	Texture class	C stock (0–30 cm) t C ha ⁻¹	Sampling Date	
Powys, Wales	52.0	-3.6	Grassland	Pre 1988	Pasture. F: '98 -'09 160kg N ha ⁻¹	Brown earth	Silt loam	76.2 \pm 9.0	10 /02/2012	
			H. Larch	1988		N F	Brown earth	Silt loam	76.3 \pm 8.4	10 /02/2012
			Sycamore	1988		N F	Brown earth	Silt loam	65.1 \pm 7.3	10 /02/2012
Moray, Scotland	57.6	-3.2	Grassland	Pre 1988	Rough Pasture. N F	Podzol	Sandy loam	94.8 \pm 22.4	14/03/2011	
			D. Birch	1998		N F	Podzol	Sandy loam	111.5 \pm 31.4	15/03/2011
			S. Birch	1998		N F	Podzol	Sandy loam	81.5 \pm 21.3	14/03/2011
			Sitka	1999		N F	Podzol	Sandy loam	136.9 \pm 44.5	15/03/2011
Moray, Scotland	57.7	-3.3	Grassland	1994	Pasture. N F	Ground-water gley	Loamy sand	39.3 \pm 8.5	17/03/2011	
			Poplar	1994		N F	Ground-water gley	Loamy sand	35.2 \pm 6.2	17/03/2011
			Alder	1996		N F	Ground-water gley	Loamy sand	38.8 \pm 8.5	18/03/2011
			Ash	1996		N F	Ground-water gley	Loamy sand	35.6 \pm 6.6	18/03/2011
North-West, England	54.0	-2.4	Grassland	Pre 1956	Rough Pasture. N F	Surface-water gley	Sandy silt loam	117.2 \pm 46.3	18/10/2011	
			Alder	1956 (1991)		N F	Surface-water gley	Sandy silt loam	122.3 \pm 25.7	18/10/2011
			Scots pine	1956 (1991)		N F	Surface-water gley	Sandy silt loam	146.8 \pm 45.7	18/10/2011
			Sitka	1991		N F	Surface-water gley	Sandy silt loam	143.4 \pm 43.7	18/10/2011
Aberdeenshire, Scotland	56.9	-2.6	Grassland	1988	Pasture. F: '02 -'09 0.97 t N ha ⁻¹	Podzol	Sandy silt loam	80.6 \pm 9.9	26/10/2011	
			Sycamore	1988		N F	Podzol	Sandy silt loam	83.1 \pm 14.5	26 /10/2011
			Scots pine	1988		N F	Podzol	Sandy silt loam	76.2 \pm 20.9	25/10/2011
			H. Larch	1988		N F	Podzol	Sandy silt loam	74.5 \pm 13.1	19/03/2012
North Lanarkshire, Scotland	55.8	-3.8	Grassland	Pre 1990	Pasture. F: Unknown	Surface-water gley	Sandy silt loam	122.9 \pm 24.1	24/11/2011	
			Alder	1990		F: Unknown	Surface-water gley	Sandy silt loam	100.8 \pm 25.0	23/11/2011
			Poplar	1990		F: Unknown	Surface-water gley	Sandy silt loam	92.0 \pm 10.7	24/11/2011
			Sitka	1990		F: Unknown	Surface-water gley	Sandy silt loam	140.9 \pm 27.8	23/11/2011

2.3.2 Laboratory Processing

Soil core sections were quartered lengthways, with quarters being allocated for different subsequent analyses; one quarter was used to derive soil C concentration and pH, and others allocated for microbial analysis and to the controlled GHG potentials laboratory incubation experiment. For further details on the soil processing methods see Keith et al. (2015).

2.3.2.1 Soil C concentration and pH analysis

Sieved (< 2 mm) oven-dried subsamples of soil were ball-milled using a Fritsch Planetary Mill (Fritsch, Idar-Oberstein, Germany) to a fine powder, and then a 100 mg sub-sample was used for the assessment of C concentration using a LECO Truspec total CN analyser (Leco, St. Joseph, MI, USA). Fresh, bulked samples were sieved to 2 mm to remove stones and roots. 10 g of bulk soil was then mixed well with 25 ml of deionised water and allowed to stand for 30 minutes, before the pH of the liquid layer was recorded using a Hanna pH 210 Benchtop Meter (Hanna Instruments, RI, USA).

2.3.2.2 Phospholipid fatty acid (PLFA) analysis

Subsamples of frozen soil were bulked at plot level (i.e. cores within plots bulked with 0–15 cm and 15–30 cm depths kept separate) and then freeze-dried prior to PLFA analysis. PLFAs were extracted using a modified Bligh-Dyer extraction (White et al., 1979). Total microbial biomass was estimated as the sum of all extracted PLFAs (Zelles et al., 1995). Bacterial biomass was estimated from the total concentration of the markers i-15:0, a-15:0, 15:0, i-16:0, 16:1 ω 7c, 16:1 ω 7t, i-17:0, a-17:0, cy-17:0, 18:1 ω 7c and 7,8, cy-19:0 (Frostegård & Bååth 1996). Fungal biomass was estimated from the concentration of the marker 18:2 ω 6 (Frostegård & Bååth 1996) and 18:9 ω 1 (Bååth, 2003). For more detailed methods of PLFA extraction and analysis see Appendix A.1.

2.3.2.3 Soil incubations (soil GHG potentials)

Fresh soil samples were used for laboratory incubations. Samples were bulked at plot level and homogenized, resulting in five samples per transition for each of the two depths (0–15 cm and 15–30 cm).

Bulk soil samples were sieved (<2 mm) and 5 g dry soil wt. equivalent weighed into 160 ml glass Wheaton bottles (Wheaton Science Products, USA). These were pre-incubated in the dark for 72 hours at 10 °C and 20 °C (target incubation temperatures for experiment) to allow equilibration (Fang & Moncrieff, 2001; Case et al., 2012). To maintain controlled moisture across all soils, water holding capacity (WHC) was adjusted to 60 % using a WHC method adapted from Ohlinger (1995) where 100 % saturation is calculated as the amount of water remaining in the soil after being saturated and left to drain for 12 h in a fully humid airspace. A water holding capacity of 60% was chosen as being approximate to field capacity (Schaufler et al., 2010) and optimum for microbial respiration (Reay et al., 2005; Vanhala et al., 2011). Following equilibration all bottles were flushed with standard compressed air for 1 minute and crimp-sealed with gas-tight septa. To compensate for gas sampling over the enclosure period, 15 ml of air was added to each bottle following closure. Bottles were then incubated at two temperatures (10 °C and 20 °C) for 7 days with headspace gas samples (5 ml) taken at 0, 24, 48 and 168 hours. Gas samples were stored in 3 ml evacuated exetainers (Labco, Lampeter, UK) for up to 2 weeks prior to analysis.

Gas samples were analysed for CO₂, CH₄ and N₂O concentrations on a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) with flame ionization detector and electron capture detector equipped with a poropack Q column operated at 60 °C with an argon carrier gas. Certified gas standards (Air Products, Crewe, UK) within the range of the samples being analysed were used to calibrate the GC. Gas fluxes (CO₂, CH₄ and N₂O) were calculated using the approach of Holland et al. (1999) by plotting the linear accumulation of each gas over the seven day enclosure period. For CO₂, CH₄ and N₂O data to be included as results a linear

response ($R^2 > 0.95$) in CO₂ concentrations with time was required. Where N₂O and CH₄ were non-linear they were still considered in the analysis as concentration changes were often negligible e.g. no flux, resulting in a low R² value. The CO₂ fluxes were also expressed per g C, in addition to being expressed by dry soil mass, in order to standardise fluxes for potential differences in soil C across land use types and transitions.

2.3.3 Statistical Methods

The influence of SRF transitions on soil C, soil pH, microbial community variables, GHG fluxes and GHG temperature response ratios was tested using linear mixed effect models with the *nlme* package in the R statistical program (R Development Core Team, 2011; Pinheiro et al., 2013). The significance of these models was examined using the *anova.lme* function. The effect of the different land uses (control and SRF types) was tested, with a fixed effect containing levels for Control, Coniferous, and Broadleaved transitions. The effect of depth and its interaction with SRF types was included in each model. To meet model assumptions, CH₄ and N₂O data were transformed prior to analysis, with data made positive by addition of the lowest value + 1 before log-transformation. For CH₄, variance was not heterogeneous across treatments and therefore data were weighted by treatment using the *varIdent* function. Data on all CO₂ fluxes and temperature response ratios were also log-transformed prior to testing.

Standardised effect sizes (Cohens' D) of change across LUC transitions were also calculated for CO₂ fluxes per g C, soil pH, total PLFA and fungal PLFA. Linear regressions between the LUC effect sizes for CO₂ flux and, soil pH, total PLFA and fungal PLFA were then undertaken to assess whether changes in soil characteristics were related to changes in CO₂ flux across transitions.

2.4 Results

2.4.1 Land use change to broadleaved and coniferous SRF

2.4.1.1 Soil C concentration and pH

Soil C concentration responded significantly to land use type ($F_{2,207} = 15.96$, $p < 0.001$) with higher soil C concentration in the coniferous soils compared to the grassland controls or the broadleaved soils (Fig. 2.1A). Although the magnitude of differences in soil C concentration varied with depth the pattern remained the same, leading to no interaction between land use and depth ($F_{2,207} = 2.78$, $p = 0.064$, Fig. 2.1A).

Land use type had a significant effect on soil pH ($F_{2,207} = 13.53$, $p < 0.001$) with, as expected, the most notable differences between the coniferous soils and both the grassland and broadleaved soils (Fig. 2.1B), and more acidic conditions measured under the coniferous land use. Little difference was observed between pH in the grassland control and broadleaved soils (Fig. 2.1B). There was also a significant effect of depth on soil pH ($F_{1,207} = 24.85$, $p < 0.001$) where, across all land use types, pH was slightly higher at 15–30 cm compared to 0–15cm depth but with no interaction between land use type and depth ($F_{2,207} = 1.22$, $p = 0.297$, Fig. 2.1B).

2.4.1.2 Microbial community (PLFAs)

Considering total PLFA data on a soil mass basis there was an effect of land use type ($F_{2,205} = 18.64$, $p < 0.001$) and depth ($F_{1,205} = 413.05$, $p < 0.001$), and an interaction between land use type and depth ($F_{2,205} = 10.54$, $p < 0.001$) (Fig. 2.1C). At 0–15 cm total PLFA in the control ($105.70 \pm 9.27 \mu\text{g g}^{-1}$ dry mass) was similar to the coniferous soils ($101.26 \pm 11.18 \mu\text{g g}^{-1}$ dry mass), but noticeably lower in the broadleaved soils ($66.35 \pm 3.22 \mu\text{g g}^{-1}$ dry mass). However, when considering total PLFA on a grams C basis the pattern changes to reflect that of CO_2 on a g C basis with lower total PLFA present in the coniferous soils compared to the grassland controls or broadleaved soils (Fig. 2.1D). The effect of land use type ($F_{2,205} = 18.64$, $p < 0.001$) and depth ($F_{1,205} = 413.05$, $p < 0.001$) were still significant but not their interaction ($F_{2,205} = 10.54$, $p = 0.193$) (Fig. 2.1D).

On a soil mass basis there was also an interaction between land use type and depth in the fungal PLFA data ($F_{2,205} = 4.36$, $p = 0.014$), with higher fungal PLFA in the coniferous soil at 0–15 cm compared to the other land use types, but no differences apparent between the land use types in the 15–30 cm soils (Fig. 2.1E). Fungal PLFA concentration was lower in the 15–30 cm soils than in the 0–15 cm soils in all land uses ($F_{1,205} = 198.14$, $p < 0.001$) but most noticeably in the coniferous soils. As with the total PLFA, considering fungal PLFA on a g C basis resulted in a switch, with lower concentrations of fungal PLFA measured in the coniferous soils compared to other land uses, although this was not significant (Fig. 2.1F). Depth was also significant ($F_{1,205} = 198.14$, $p < 0.001$) but not the interaction between land use and depth ($F_{2,205} = 4.36$, $p = 0.364$) (Fig. 2.1F). Bacterial PLFAs followed the same pattern as total PLFA with differences between the land uses ($F_{2,205} = 10.79$, $p < 0.001$) decreasing from control > coniferous > broadleaved at 0–15 cm depth, and from control > broadleaved > coniferous at 15–30 cm depth (data not shown).

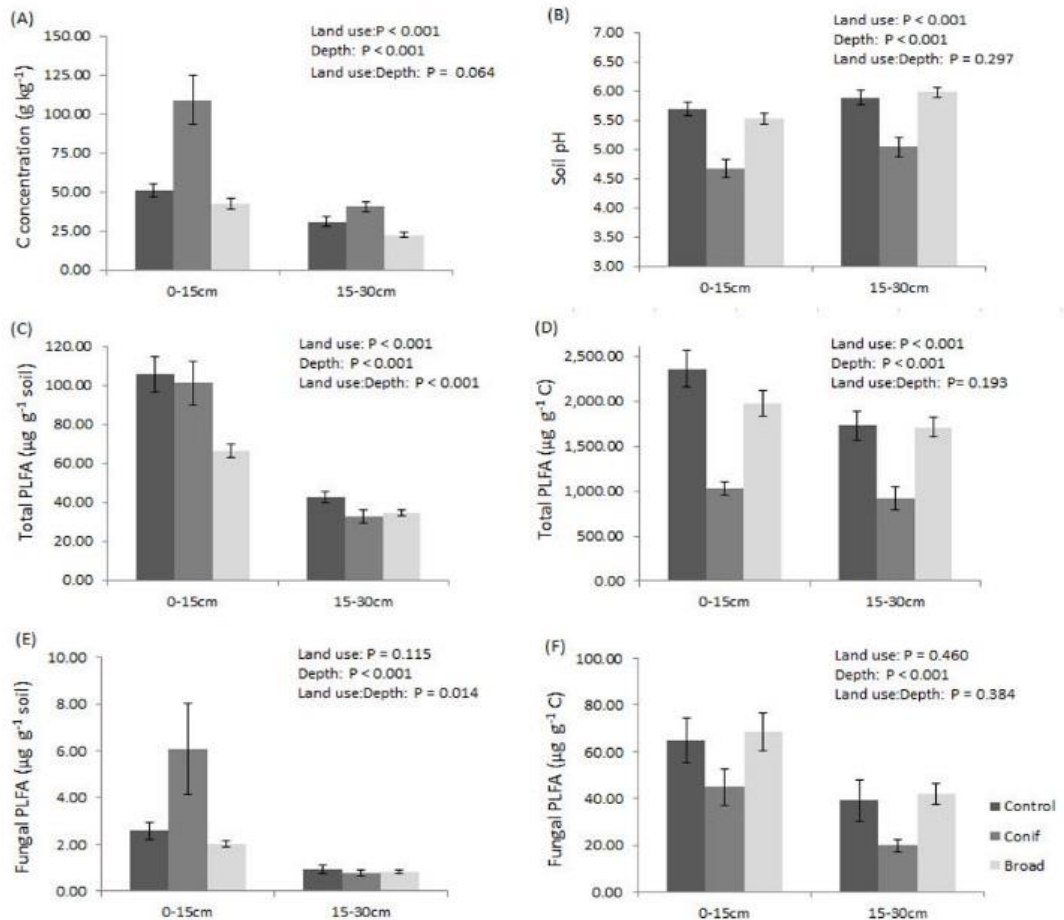


Figure 2.1 Measured (A) Soil carbon concentration (g kg⁻¹), (B) Soil pH, (C) Total PLFA (μg g⁻¹ soil), (D) Total PLFA (μg g⁻¹ C), (E) Fungal PLFA (μg g⁻¹ soil) and (F) Fungal PLFA (μg g⁻¹ C) from soils under different land uses (control, broadleaved and coniferous) and different depths. Data in (C) and (E) are based on soil dry weight. Note scales are not consistent; error bars represent standard error.

2.4.1.3 GHG Fluxes

An effect of land use type ($F_{2,207} = 15.41$, $p < 0.001$) on CO₂ flux on a soil mass basis was found, and fluxes were lower in broadleaved soil than in either coniferous land uses or grassland control. There was little difference in soil CO₂ flux between control and coniferous land use and no interaction between land use and depth, although fluxes were lower in the 15–30 cm layer than in the 0–15 cm layer ($p < 0.001$, Fig. 2.2A). However, when considering soil CO₂ flux on a soil C basis the output is considerably

different. Although the effects of land use type ($p = 0.028$), depth ($p < 0.001$) and the interaction between land use and depth ($p = 0.136$) were consistent, CO₂ fluxes are now considerably lower in coniferous soils compared to the grassland control and broadleaved land use. The CO₂ flux was similar between grassland control and broadleaved land uses at 0–15 cm when accounting for soil C concentration (Fig. 2.2B).

The temperature response ratio of soil CO₂ flux was greater under coniferous than under broadleaved or grassland land uses at both depths, though not significantly so. The coniferous and grassland land uses demonstrated a trend towards higher temperature responses ratios at 15–30 cm depth compared to 0–15 cm, this was not the case for the broadleaved land use where the temperature response ratio was slightly lower at 15–30 cm compared to 0–15 cm. The temperature responses followed the same pattern across land use types on a soil mass and g C basis (Table. 2.2).

CH₄ flux was mostly negative and very small (range: -0.58 – 0.20 ng CH₄ g⁻¹ soil dwt hr⁻¹) across all land uses and depths, indicating that CH₄ was being consumed under all species (Fig. 2.2C). Although greatest consumption was measured from broadleaved soils and the lowest in coniferous soil, there was no significant effect of land use on CH₄ flux ($F_{1,207} = 0.148$, $p = 0.862$). There was an effect of depth on CH₄ flux ($F_{1,207} = 18.46$, $p < 0.001$) with lower uptake measured at 15–30 cm depth across all land uses but no interaction between land use and depth ($F_{2,207} = 1.78$, $p = 0.171$).

Soil N₂O flux rates were also very low, ranging from -0.16 – 0.05 ng N₂O-N g⁻¹ soil dwt hr⁻¹, and there was no difference between the land uses (Fig. 2.2D). There was a depth effect ($F_{1,207} = 22.72$, $p < 0.001$) and higher flux rates were measured in the 0–15 cm soils but there was no interaction between land use and depth ($F_{2,207} = 2.62$, $p = 0.075$).

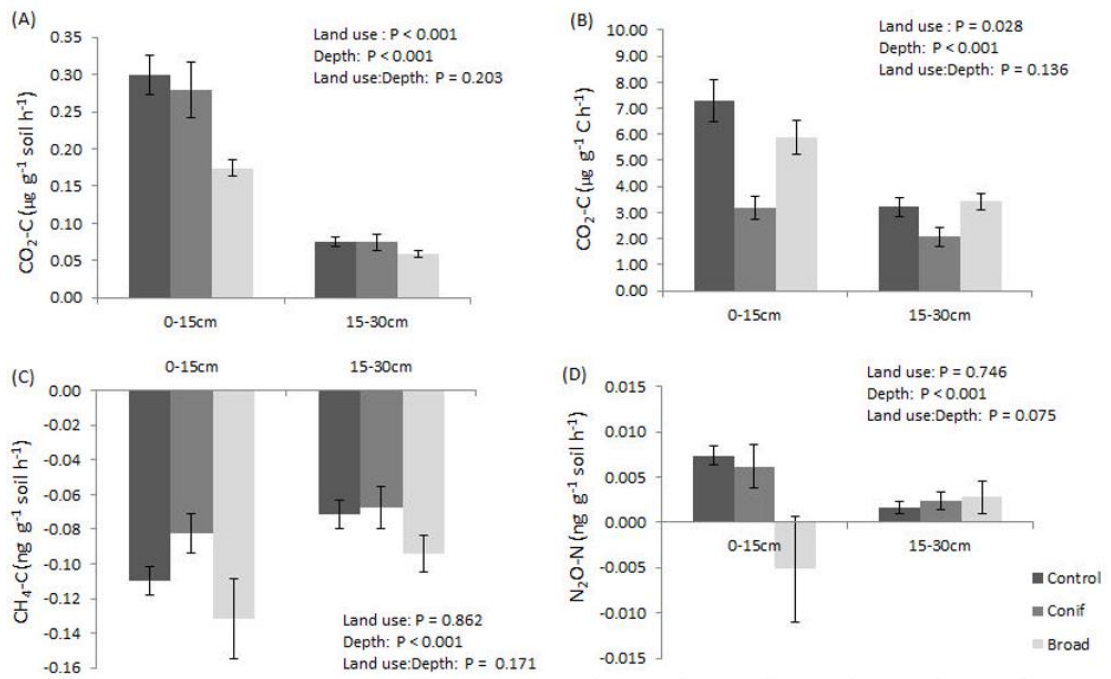


Figure 2.2 Potential fluxes of (A) CO_2 ($\mu\text{g g}^{-1} \text{ soil hr}^{-1}$), (B) CO_2 ($\mu\text{g g}^{-1} \text{ C hr}^{-1}$), (C) CH_4 ($\text{ng g}^{-1} \text{ soil hr}^{-1}$), and (D) N_2O ($\text{ng g}^{-1} \text{ soil hr}^{-1}$) from soils under different land uses (control, coniferous and broadleaved) and different depths. Fluxes in (A), (C) and (D) are based on soil dry weight. Note scales are not consistent; error bars represent standard error.

Table 2.2 Soil CO₂ flux temperature response ratio's (ratio between CO₂ flux at 10° C and 20° C) and summary statistics from linear mixed effect models on the effect of land use type (grassland control, coniferous and broadleaved), depth and their interaction on CO₂ fluxes in soils. CO₂ (µg g⁻¹ soil h⁻¹) data are based on soil dry weight. Values represent means ± standard error.

Land Use/Depth	CO ₂	CO ₂
	(µg g ⁻¹ soil h ⁻¹)	(µg g ⁻¹ C h ⁻¹)
Grassland 0-15 cm	2.88 (± 0.35)	2.83 (± 0.32)
Grassland 15-30 cm	3.28 (± 0.36)	3.40 (± 0.45)
Coniferous 0-15 cm	3.27 (± 0.46)	3.21 (± 0.43)
Coniferous 15-30 cm	3.57 (± 0.51)	3.70 (± 0.63)
Broadleaved 0-15 cm	2.72 (± 0.86)	2.75 (± 0.09)
Broadleaved 15-30 cm	2.61 (± 0.96)	2.57 (± 0.08)
<i>Mixed model fixed effect</i>		
Land Use	P = 0.874	P = 0.862
Depth	P = 0.719	P = 0.790
LU: Depth Interaction	P = 0.131	P = 0.066

2.4.1.4 Effect sizes across land use change transitions

Linear regressions were performed on effect sizes of soil characteristics and CO₂ fluxes per g C across grassland to SRF transitions to determine the variables in which changes were most strongly related. There were positive relationships between LUC effect sizes of soil pH and CO₂ flux (0–15 cm: F = 4.0, p = 0.067, R² = 0.176; Both depths: F = 4.8, p = 0.038, R² = 0.115; Fig. 2.3A). Stronger positive relationships, however, were shown between LUC effect sizes of both total and fungal PLFA, and CO₂ flux. Total PLFA effect sizes had a significant relationship with CO₂ flux effect sizes considering only 0–15 cm samples (F = 117.2, p < 0.001, R² = 0.893) and both depths (F = 220.2, p <

0.001, $R^2 = 0.887$), with the slope of the relationship virtually identical (Fig. 2.3B). Likewise, fungal PLFA effect sizes also had a significant relationship with CO₂ flux effect sizes considering only 0–15 cm samples ($F = 8.9$, $P < 0.001$, $R^2 = 0.378$) and both the 0–15 cm and 15–30 cm depths ($F = 12.8$, $p < 0.001$, $R^2 = 0.312$), with similar slopes (Fig. 2.3C).

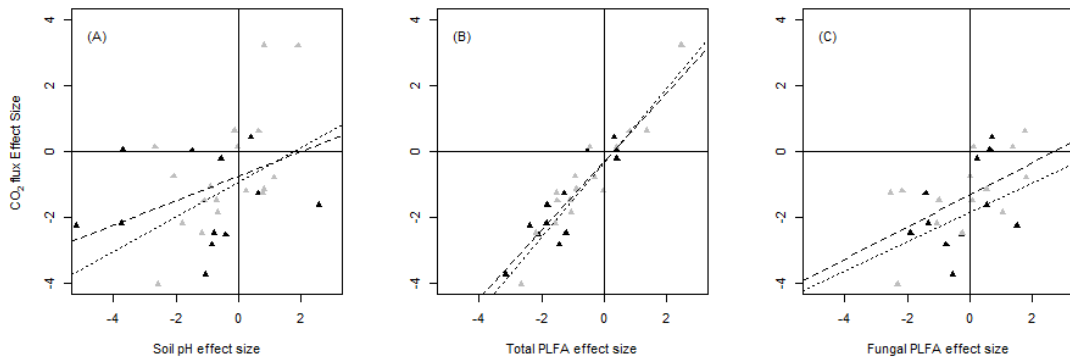


Figure 2.3 The relationship between Land Use Change (LUC) transition effects on soil CO₂ (on a mass of C basis) potential flux, pH and soil microbial community measures. Effect sizes of (A) pH and CO₂ potential flux, (B) Total PLFA and CO₂ potential flux and (C) Fungal PLFA and CO₂ potential flux. The effect size of LUC transitions measured as standardised effect sizes, Cohen’s D. Black and grey symbols represent samples from 0–15 cm and 15–30 cm, respectively; dashed and dotted lines represent significant relationship between effect sizes for 0–15 cm samples only and both depths, respectively.

2.5 Discussion

Utilising laboratory soil incubations under standardised temperature and moisture conditions, we examined potential GHG fluxes in soils from LUC transitions to SRF. This study demonstrated clear differences in CO₂ flux but not N₂O or CH₄ fluxes between grassland and SRF land uses and, in line with a previous study at these sites looking at soil C stocks (Keith et al., 2015), distinctions between transitions to broadleaved and coniferous tree species were also observed. Such laboratory approaches are important to disentangle different factors influencing soil respiration and C turnover and they allow exploration of the direction and magnitude of relationships (Schaufler et al., 2010). However, they are not without their limitations due to the unnatural and standardised conditions. Short-term incubations, such as those carried out in this study, only measure the initial response of soil GHG processes to changes in temperature and therefore may not reflect the effect of long-term changes in temperature (Li et al., 2012). Soil is also disturbed during sample preparation as a result of sieving, homogenising and removing roots, and this may alter the soil structure and environment resulting in artificial aeration of soils which can affect soil atmosphere GHG exchange (Reichstein et al., 2005; Schaufler et al., 2010). Nonetheless, where reductionist laboratory experiments are required, using fresh sieved soils has been recommended as having the least impact on microbial communities and C cycling processes (Thomson et al., 2010).

2.5.1 Differences between transitions to broadleaved and coniferous species

Soil GHG fluxes are influenced by many natural and anthropogenic factors such as soil type, pH, nutrient status, forest type, stand age and land management (Morison et al., 2012), and therefore measurements are generally very variable reflecting the diversity of these factors. In this study, there were differences in CO₂ flux expressed by soil mass between coniferous and broadleaved soils, with no apparent change under coniferous tree species. However, once CO₂ flux had been expressed per g C to account for differences in soil C between land use type and across transitions, LUC

from grassland to coniferous SRF resulted in greatly reduced CO₂ fluxes while in the broadleaf SRF CO₂ fluxes were generally unchanged. A reduction in CO₂ flux may be expected to be associated with lower decomposition rates and hence increased soil C concentration. Indeed, the reduced CO₂ fluxes in transitions from grassland to conifers (this study) and increased soil C concentration and C stocks (Keith et al., 2015) suggest that there is good potential for enhanced C storage under coniferous SRF as a bioenergy crop. The similar CO₂ fluxes and soil C concentration under grassland controls and broadleaved SRF suggests that, while there is less potential for soil C storage under this type of SRF, its overall effect will not be negative. This is supported by previous analysis of soil C in the same SRF transitions which showed that broadleaved species contained similar stocks of soil C to controls (Keith et al., 2015).

Other studies have also found mixed outcomes with respect to differences between conifer and broadleaved species. Brüggemann et al. (2005) found a similar pattern in a laboratory experiment measuring soil respiration from under different tree species with highest rates being measured from spruce soils in both the organic layers and A_h horizons compared to four deciduous species. In contrast to the results of this study and those of Brüggemann et al. (2005) soil respiration rates were found to be ~10 % lower in coniferous stands compared to adjacent deciduous stands in a review by Raich and Tufekciogul (2000). Results of some studies have been variable, for example Schaufler et al. (2010) looked at the effect of land use on soil GHG emissions under controlled laboratory conditions and discovered that tree species had variable effects on GHG flux rates, with CO₂ flux declining in the order of beech > pine > oak > spruce. Others have found no differences in CO₂ fluxes/respiration rates between coniferous and deciduous species types (Ladegaard-Pedersen et al., 2005; Subke et al., 2006; Wunderlich et al., 2012; Vesterdal et al., 2012). These variable findings suggest that how CO₂ flux is expressed may be important to the outcome determining whether there are broad differences between coniferous and broadleaved tree species.

In this study the temperature sensitivity of CO₂ flux (for both soil mass and g C basis) was higher, though non-significant, in the coniferous soils at both depths, and lowest in broadleaved soils (Table. 2). In the grassland and coniferous soils the temperature response of respiration also increased at depth. C-rich coniferous soils are formed from high volumes of lignin-rich recalcitrant needle litter which decomposes slowly, leading to the formation of a thick C-rich humic layer (Morison et al., 2012). Mixed findings exist regarding the response of recalcitrant C to increased temperature (Chen et al., 2010) but generally it is thought that temperature sensitivity increases with recalcitrance of a substrate (Craine et al., 2010) as more energy is required for the enzymatic decomposition of recalcitrant substances than more labile substances (Davidson & Janssens, 2006).

Differences in N₂O fluxes were not significant but values suggested a potential for N₂O consumption in the broadleaved compared to N₂O production in the other land uses. The trend of higher emissions under coniferous compared to broadleaved species may in part be attributed to soil N availability, though this was not measured. Soil N availability is a key driver of soil N₂O emissions and it is known that coniferous stands receive more N via deposition than adjacent deciduous stands (Christiansen & Gundersen, 2011; Hansson et al., 2011). However, other studies indicate there may be higher N₂O production from broadleaved than coniferous forest soils (Ambus et al., 2006; Pilegarrrd et al., 2006; Ullah et al., 2008) which highlights the complexity surrounding the multiple interacting drivers of soil N₂O production and consumption (Butterbach-Bahl et al., 2013). CH₄ was consumed under all land uses in this study but there were no significant differences in consumption rates. This is consistent with the knowledge that aerobic forest soils and grasslands are important terrestrial sinks for CH₄ (Borken et al., 2003; Menyailo & Hungate, 1998). There was a trend towards greater methane consumption in broadleaved soils which follows the work of others. Our results showed that CH₄ oxidation rates were higher in the surface 0–15 cm soils which supports the notion that methanotrophy in forests has a sub-surface maximum in the upper soil layers (Hütsh, 1998; Adamsen & King, 1993).

Soil physico-chemical properties and soil microbial community characteristics were also found to differ between coniferous and broadleaved land uses following conversion to SRF. As expected soil acidity increased in the coniferous soils, but there was no change in pH between the control grassland and broadleaved soils. It is well known that growing conifers affects soil pH, by creating more acidic soil conditions due to the poorer quality of their litter inputs (Wedderburn & Carter, 1999; Peterken, 2001; Morsion et al., 2012). These acidic conditions created under coniferous tree species can inhibit microbial activity and reduce decomposition rates leading to potential increases in soil C (Morison et al., 2012). In this study, greater C concentrations were measured in the coniferous soils compared to the grassland control and broadleaved soils and, once PLFAs had been expressed per g C to account for differences in soil C, a reduction in total PLFA. However, biomass is not necessarily a direct measure of activity but related to a range of other factors including microbial community composition (Bardgett et al., 2008). Differences in microbial composition were also observed with higher fungal PLFA concentrations per g C in broadleaved soils compared to both grassland control and coniferous soils. Other authors have observed greater fungal PLFA under coniferous species compared to broadleaved species (Hackl et al., 2005). In contrast Priha et al. (2001) measured higher total PLFA and fungal PLFA in birch soil compared to pine or spruce soils. Nevertheless, these differences in soil physico-chemical and microbial characteristics may be important drivers of the GHG fluxes observed in this study.

2.5.2 Links between respiration and microbes across LUC transitions

In order to assess which variables were most strongly related to changes in CO₂ flux across LUC transitions in this study, effect sizes were assessed to determine whether changes in CO₂ flux were related to changes in soil pH and microbial community characteristics. While the effect sizes of soil pH significantly related to effect sizes of CO₂ flux, R-squared values were relatively low. In contrast, the positive relationships found between PLFA effect sizes and CO₂ effect sizes were stronger. In particular, reductions in CO₂ flux were strongly associated with reductions in total PLFA across

transitions. These data suggest that shifts in microbial communities across these LUC transitions have a greater impact than the direct effect of changes in soil pH.

Changes in the microbial communities observed due to LUC to SRF may be linked to impacts on microclimate and/or litter and root inputs (Prescott & Grayston, 2013). A study by Vesterdal et al. (2012) found different soil C turnover rates among six tree species (beech, lime, spruce, maple, ash, oak) despite having similar quantities of aboveground litterfall; the authors suggest that tree species have the greatest impact on soil C stocks via the indirect effects of litter quality on microbial activity and decomposition rates. Although not measured, the tree species in this study are likely to have had similar differences in litter quality. The quality of tree inputs from litter and rhizodeposition also vary due to differences in plant chemistry between coniferous and broadleaf species which in turn influences soil microbial composition and more specifically the relative abundance of fungi and bacteria. Clear differences in the abundance of soil fungal and bacterial PLFAs were observed in this study between land uses, with higher concentration per g C of both measured in the broadleaved compared to the coniferous soils. Fungi are considered to promote slower decomposition cycles with increased nutrient retention (Wardle et al., 2004) and are important for degrading more complex substrates compared to bacteria (Rousk & Bååth, 2011). As in this case, differences in the composition of the microbial communities (e.g. the relative abundance of fungi and bacteria) have been shown to influence CO₂ fluxes from soil in other studies (Kant et al., 2011; Whitaker et al., 2014).

2.6 Conclusion

SRF is a growing bioenergy land use in temperate climates which has the potential for reduced GHG emissions and increased C storage but understanding of its effects on these factors is limited. Comprehensive data on C changes associated with LUC to bioenergy crops are essential to be able to assess their sustainability. This study provides evidence that LUC to SRF for bioenergy could lead to GHG savings through reduced C loss via soil respiration. These findings strongly suggest that careful consideration should be given to the selection of SRF species in order to optimise soil C storage and GHG reduction.

Changes in land use and management has significant impacts on the microbial community, and there is a challenge to better understand the effect of LUC to bioenergy on GHG fluxes and their relationship with the soil microbial community. Here, lower soil CO₂ fluxes under SRF appeared to be associated with reductions in microbial biomass and changes in broad community composition (i.e. bacteria and fungi). Consequently, both direct and indirect effects of planting SRF on the soil microbial community may be important mechanisms by which GHG emissions are reduced.

Chapter 3: Differential effects of tree species considered for SRF on soil GHG fluxes

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Declaration of contribution

This field based study was planned and carried out by Kim Parmar with fieldwork assistance from Dr Aidan Keith, Dr Rachel Marshall and Andrew Fitton. The construction of litter bags and all laboratory analyses were carried out by Kim Parmar, with the exception of the $\text{NH}_4^+/\text{NO}_3^-$ measurements which were made by Benjamin Jackson. Data were analysed with help from Dr Aidan Keith and the chapter was written by Kim Parmar and reviewed and commented on by Dr Aidan Keith, Dr Niall McNamara and Dr Saran Sohi.

3.1 Abstract

Short Rotation Forestry (SRF) could be used to meet biomass requirements in the future. Although not currently widespread in the UK, a suite of species is under consideration for SRF including a range of coniferous and broadleaved species. It is important to identify and understand tree species effects on soil greenhouse gas (GHG) fluxes so that informed decisions can be made regarding suitable SRF to ensure genuine GHG emissions savings in the future.

Plots at the Gisburn Experimental Forest site, north-west England, were studied over 16 months to investigate the influence of LUC from grassland to tree species that could be grown as SRF, on soil GHG fluxes (CH_4 & N_2O) and net CO_2 flux from soil and understorey. GHG's were sampled from stands of common alder (*Alnus glutinosa*), Scots pine (*Pinus sylvestris*), Sitka spruce (*Picea sitchensis*) and rough grassland on a monthly basis from May 2013 to August 2014.

Lower CH_4 and lower net CO_2 , and higher N_2O emissions were recorded under SRF compared to the grassland. There were also significant differences between tree species in net CO_2 and N_2O fluxes which switched through time. There was an interactive effect of tree species and sampling time for N_2O fluxes; common alder treatments exhibited greatest N_2O emission from May-July but Sitka spruce had greatest N_2O emission from August onwards. Net CO_2 fluxes were driven largely by soil temperature. Random spatial effects explained a large proportion of the variation in soil CH_4 flux. Whereas N_2O flux was driven by habitat and the interaction between habitat and water table depth.

Using this method it has been demonstrated that LUC from grassland to SRF can lead to lower soil emissions of CH_4 , indicating a potential benefit to C sustainability, however, it has also been demonstrated that increases in N_2O must be considered with respect to climate abatement. The interactive effects between tree species and season on N_2O emissions also suggest that SRF species must be considered carefully

for future GHG mitigation. Future work should focus on whether these patterns are consistent across other soil types and geographical zones.

3.2 Introduction

Land use change (LUC) to bioenergy crops is likely to be an important component of the strategy to meet growing energy demands whilst meeting UK greenhouse gas (GHG) emission reduction targets and European renewable energy targets (DECC, 2012). Compared to other renewable energy sources, bioenergy is more cost-effective making it an attractive option to meet targets (POST, 2012). To date, there has been strong environmental rationale for supporting bioenergy as a fossil fuel alternative as it has the potential to deliver genuine GHG savings (Hastings et al., 2009; Rowe et al., 2009; Thornley et al., 2015) but the belief that it is “carbon neutral” due to the C accumulated during biomass growth being released during burning is now recognised as an oversimplification (Field et al., 2008; Ter-Mikaelian et al., 2015). In fact, pre-harvest emissions could potentially offset any C savings via reduced fossil fuel use and therefore any loss of C could increase the risk of bioenergy crops becoming carbon positive (Don et al., 2011). There is a clear need to quantify the effects of planting perennial bioenergy crops on soil C and the GHG balance (Don et al., 2011; Keith et al., 2015) and to date there are few, if any, long-term data sets available.

Short-Rotation Forestry (SRF) is a perennial woody crop which has potential as a bioenergy crop. SRF differs from conventional commercial forestry in that high density plantations of fast-growing single stemmed broadleaved or coniferous species are grown on shorter rotational lengths and harvested for biomass when a breast height of 10–20 cm has been reached (Hardcastle et al., 2006; McKay, 2011). Previous land use and tree type could impact the direction and magnitude of the effect of LUC to woody bioenergy on soil C accumulation and GHG exchange (Guo & Gifford 2002; Laganriere et al. 2010; Keith et al., 2015). A meta-analysis carried out by Harris et al. (2015) to quantify the effects of LUC to second generation bioenergy crops, found that changes in soil C were dependent on the original land use (arable, forest or grassland). Harris et al. (2015) found that LUC from arable crops to bioenergy crops resulted in increased soil C, LUC from forestry to bioenergy resulted

in decreased soil C, and LUC from grassland to bioenergy showed variable results which were transition dependent. The authors found insufficient data to carry out the meta-analysis for soil GHG emissions, but reported trends showed a reduction in soil GHG emissions following LUC from arable to bioenergy, a general increase in GHG emissions following LUC from grassland to bioenergy and a clear increase in GHG emissions following LUC from forestry to bioenergy. However, there was very limited data available on grassland transitions, and no data available on LUC to SRF (Harris et al., 2015).

SRF species type could have an impact on the direction and magnitude of soil GHG emissions and soil C storage potential, mainly due to differences in tree species inputs and effects on soil properties, and particularly in regard to coniferous and broadleaved species. A recent study by Keith et al. (2015) measuring changes in soil C following LUC from grasslands to SRF across 11 sites in the UK, found greater soil C accumulation under coniferous species compared to broadleaved species. Transitions to eucalypts showed a trend, albeit insignificant, towards soil C loss whereas transitions to broadleaved species resulted in no change from the agricultural land use but displayed the most variable response suggesting species related effects may be at play. A meta-analysis carried out by Guo and Gifford (2002) examining LUC effects on soil C, using 74 individual studies across 16 countries, also found that broadleaf plantations had little effect on soil C change while conifers reduced soil C by up to 12%. However, a study by Laganière et al. (2010) found broadleaved plantations to have a positive effect on soil C following transition from agricultural land to forestry compared to pine and Eucalyptus species. Vesterdal et al. (2013) conducted a review on tree species effects on soil C stocks in temperate forests and found many contrasting results and suggest that this is due to the existence of species-specific and site-specific influences. Due to these contrasting and variable results, a study carried out at a single site with a range of tree species, similar to common garden experiments by Reich et al. (2005), Trum et al. (2011) and Vesterdal

et al. (2008, 2012), could help to clarify species-specific effects on soil C and soil GHG emissions following LUC to SRF.

Tree species directly affect soil physical, chemical and biological properties due to differences in the quantity, quality (C/N ratio, N content, lignin) and decomposition rates (faster in broadleaved species compared to coniferous) of litter inputs, and as a result of differences in root litter inputs and root architecture, all of which in turn influence nutrient turnover rates, soil structure, soil moisture content and GHG exchange (Hansson et al. 2011; Vesterdal et al., 2008). Ambus and Zechmeister-Boltenstern (2007) reported increased nutrient turnover rates and microbial activity from broadleaf soils compared to coniferous soils. Changes in water table levels and subsequent soil aeration can have a significant effect on soil GHG emissions (Ball et al., 2007). Afforestation of organic soils in particular can cause an increase in water table depth, leading to a decrease in soil CH₄ flux (Hughes et al., 1999). Jungkunst et al. (2004) found highest N₂O emissions from soils with intermediate aeration, as a consequence of having water tables in the depth range of 15-35 cm.

European forest ecosystems are predicted to act as significant GHG sinks (Schulze et al., 2010) as afforestation is seen as a means of mitigating rising CO₂ levels in the atmosphere by means of increased C storage in soil and biomass (Christiansen & Gundersen, 2011). Trees absorb CO₂ from the atmosphere via photosynthesis, much of this is released again via heterotrophic and autotrophic respiration and the remainder is allocated to leaves, roots, stems and branches which can lead to above and belowground C storage (Morison et al., 2012). Soil C stocks are controlled by the balance between inputs (via litterfall, root exudates) and the outputs via microbial decomposition and subsequent soil respiration (Vesterdal et al., 2012). Although CO₂ emissions contribute the largest percentage to global GHG emissions afforestation can also affect the dynamics of soil/atmosphere CH₄ and N₂O exchange. These two gases are of particular importance due to their higher global warming potentials (GWPs) (34 and 298 for CH₄ and N₂O respectively) compared to CO₂.

Soil CH₄ fluxes result from the microbial processes of methanogenesis (production), which generally occurs under anaerobic conditions, and methanotrophy (consumption) which generally occurs in aerobic conditions. Afforestation is known to generally promote methanotrophy (Smith et al., 2000) as forest soils are usually quite porous and therefore more aerated which provides a soil environment more favourable for CH₄ consumption (Christiansen & Gundersen, 2011), although CH₄ uptake can be suppressed in soils where there is high available N (Reay & Neadwell, 2004; Reay et al., 2005). In forest systems where the soils have a high organic content and high water table levels, significant CH₄ emissions may be produced via methanogenesis despite increased soil porosity (Don et al, 2011). Some studies have shown greater CH₄ uptake from broadleaved soils compared to coniferous soils and attributed this to reduced diffusion potential in coniferous soils due to the presence of a deep soil organic layer (Hudgens & Yavitt, 1997; Butterbach-Bahl & Papen, 2002; Borken et al., 2006; Degelmann et al., 2009).

Nitrous oxide (N₂O) from soil is produced via two primary pathways, nitrification and denitrification (Khalil *et al.*, 2004; Wrage *et al.*, 2005; Gillam *et al.*, 2008). Nitrification is dominant under aerobic conditions, whereas under increasingly anaerobic conditions denitrification is the dominant pathway (Bateman & Baggs, 2005). Nitrous oxide production is also constrained by temperature, inorganic-N content, pH and the form and concentration of labile C (Hofstra & Bouwman, 2005). Temperate forest soils may be a significant source of N₂O, estimated to emit up to 8.07 kg N₂O-N ha⁻¹ yr⁻¹ (Dalal and Allen, 2008). The effect of forest type (coniferous or broadleaved) on N₂O emissions is uncertain (Christiansen and Gundersen, 2011) and soil N status rather than forest type may be a greater driver of this flux (Liu and Greaver, 2009).

It is important to quantify soil GHG fluxes in order to evaluate sustainability and viability of LUC to SRF and to determine which tree species offer the best GHG savings. However, there are limited data regarding soil-atmosphere GHG exchange following LUC to SRF. For example, Keith et al. (2015) estimated changes in soil and

aboveground C across a range of SRF transitions but didn't examine changes in soil GHGs. Earlier work (Chapter 2) using the same transitions as Keith et al. (2015) showed differences in laboratory GHG potentials between SRF and grassland, and differences between tree types. Here, we describe *in situ* GHG fluxes over a 16 month period under different SRF species (common alder, Scots pine, and Sitka spruce) and make comparisons to the original ungrazed rough grassland land use. Whilst net CO₂ flux from soil and understorey is reported here, we are unable to separate contributions from different components of respiration [Soil respiration was measured in mesocosms from Gisburn habitats in the absence of plants in Chapter 4]. We hypothesised that 1) LUC from rough ungrazed grassland to SRF would reduce soil CH₄ emissions through lower water table depth and conditions more favourable for soil CH₄ consumption, and increase N₂O emissions because of increased N availability and lower water table, 2) there would be differences in soil GHG fluxes between tree species, and 3) different soil and environmental variables will be the key drivers of soil GHG fluxes under different tree species. Overall, we would like the information derived from this work to be used in tree species selection for SRF that provides the greatest soil GHG savings.

3.3 Materials and methods

3.3.1 Site description

This field study was carried out at Gisburn Forest experimental site in the NW England (54° 1' N; 2°22' W) which was established in 1955 and is managed by the Forestry Commission. This long-term, fully replicated and randomised plot experiment (Fig. 3.1) is located on a gentle southwest facing slope and ranges in altitude from 260-290 m above sea level. The underlying geology consists of Carboniferous grits, shales and sandstones overlain by acid clayey till. Soils are predominantly cambic stagnogleys and stagnohumic gleys (Avery, 1980), with variation in depth to clay horizon between experimental blocks (Moffat and Boswell, 1990). The mean annual rainfall (1981-2010) is 1294 mm with an average 168 days of rain per year (Met Office Climate Data for Stonyhurst Weather Station, Lancashire).

Due to the combined effect of high rainfall and the presence of clayey soils the site is poorly drained with a projected soil moisture deficit of approximately 100 mm (Mason & Connolly, 2014). The original Gisburn experimental design consisted of three blocks containing pure and mixed 0.2 ha plots of Sessile Oak (*Quercus petraea*), Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), common alder (*Alnus glutinosa*) with a rough ungrazed grassland control (*Festuca-Agrostis* with *Nardus stricta*, Fig. 3.2C), and was fully fenced to keep deer and rabbits out (Fig 3.1). For the purposes of this study only the pure plots of Scots pine, Sitka spruce, common alder and the grassland controls were used from each block (the use of the term ‘habitat’ throughout this chapter refers to the different land covers including Scots pine, Sitka spruce, common alder and grassland). The site was clear felled in 1989 due to wind throw and replanted in the original design in 1991, but with the addition of Sitka spruce (*Picea sitchensis*). Prior to replanting in 1991 the plots were ploughed, resulting in a local microtopography consisting of a planting ridge with a hollow (trough) on one side and an undisturbed flat area on the other. No fertilisers have been applied to the soils at or since establishment. Weed control using propyzamide and glyphosate was carried out between 1992 and 1996 (Mason & Connolly, 2014). Understorey cover varied in the SRF plots, with almost full grass/moss cover in the common alder, ~ 70% grassy cover in the Scots pine, but no ground cover in the Sitka spruce plots (Fig. 3.2A, B, D, E & F). Soil properties for different habitats are summarised in Table 3.1.

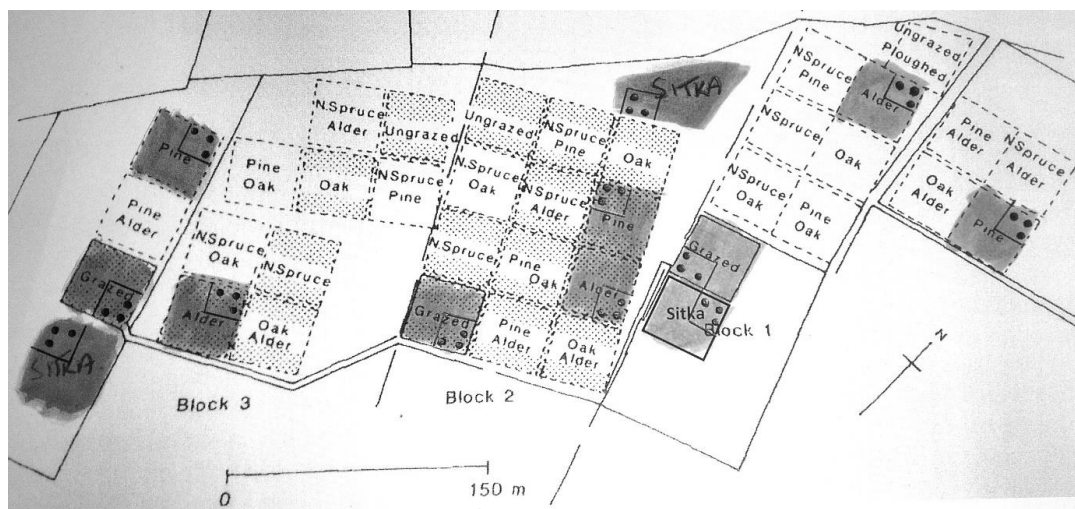


Figure 3.1. Map of Gisburn Forest experimental site in Lancashire. Dashed squares are individual plots; black dots indicate GHG static chamber locations within each sampled plot. Sitka spruce plots have been added by hand as there were not part of the original design.

Table 3.1. Summary of soil properties under rough grassland, Scots pine, Sitka spruce and common alder at the Gisburn Forest experimental site. Bulk density, C, N, C:N ratio and C stock data were calculated from 15 cm deep cores taken as part of the Ecosystem Land Use Modelling dataset which was gathered in 2012, n = 15 (Keith et al., 2015). Values for pH and organic layer depth were calculated from cores collected monthly over the duration of this field study, n=108. Concentrations of ammonium (NH₄⁺) and nitrate (NO₃⁻) were calculated from soil cores collected during this field study in May '13, August '13, November '13, February '14, May '14, n = 108. Values in parentheses represent standard errors.

	grassland	Scots pine	Sitka spruce	common alder
Bulk Density	0.45 (± 0.06)	0.47 (± 0.07)	0.43 (± 0.10)	0.45 (± 0.06)
pH	4.68 (± 0.15)	4.12 (± 0.28)	4.28 (± 0.16)	4.00 (± 0.15)
Carbon (%)	7.61 (± 1.36)	13.72 (± 2.25)	17.80 (± 4.62)	11.38 (± 0.96)
Nitrogen (%)	0.57 (± 0.08)	0.74 (± 0.08)	0.80 (± 0.15)	0.75 (± 0.05)
C:N Ratio	12.93 (± 0.85)	18.10 (± 1.26)	20.69 (± 1.96)	15.15 (± 0.37)
C Stock (t C ha ⁻¹) (0-30 cm)	117.20 (± 46.30)	146.80 (± 45.70)	143.40 (± 43.70)	122.30 (± 25.70)
GMC (%)	49.55 (± 4.70)	51.22 (± 0.02)	33.31 (± 1.89)	41.68 (± 4.70)
Organic layer depth (cm)	2.32 (± 0.13)	2.76 (± 0.13)	4.35 (± 0.18)	2.22 (± 0.15)
NO ₃ ⁻ -N (mg kg ⁻¹)	0.83 (± 0.16)	0.73 (± 0.16)	0.81 (± 0.29)	1.94 (± 0.26)
NH ₄ ⁺ -N (mg kg ⁻¹)	22.25 (± 2.82)	20.29 (± 2.43)	23.24 (± 3.46)	17.75 (± 1.71)

3.3.2 Methods

3.3.2.1 GHG fluxes

Soil GHG measurements (CH₄ and N₂O) and net CO₂ flux from soil and understorey were made using the static opaque chamber method adapted from Livingston and

Hutchinson (1995). The net CO₂ flux includes aerobic and anaerobic decomposition processes, respiration of other soil organisms, total dark respiration of ground vegetation and root respiration of trees where present (Yamulki et al. 2013). Three PVC chambers (40 cm w x 20 cm h) were installed to a depth of ~ 5 cm in each of the Sitka spruce, Scots pine (Fig. 3.2A), common alder and rough grassland plots within each of the three blocks (total 36 chambers) in May 2013. Within each of the 45 x 45 m plots the central quadrant, representing 20% of the total plot area, was excluded from sampling at the request of Forest Research. One corner of each plot (representing 25% of total plot area) was randomly selected to position GHG chambers (Fig. 3.1). As the corners of each plot were directed approximately to the cardinal compass points one of these were randomly selected for each plot using the *sample* function in the *base* package in R (R Development Core Team, 2011). At each plot corner, the first chamber was positioned 8 m from the selected corner to avoid edge effects, and a second and third chamber were positioned 10 m away from, and perpendicular to, the corner chamber (Fig. 3.1). All of the chambers remained in the soil for the 18 month duration of field sampling. Following chamber installation a settling period of one week was allowed before first sampling took place. Sampling took place approximately every four weeks from May 15th 2013 to August 13th 2014 by enclosing each chamber with a reflective aluminium lid fitted with a rubber seal to prevent leakage and a self-sealing rubber septa. Between the hours of 11:00 and 14:00 on the day of sampling, 10-ml headspace gas samples were collected using a needle and syringe every 15 minutes over a 45 minute enclosure period into pre-evacuated 3-ml exetainers (Labco, Lampeter, UK). We acknowledge that GHG fluxes can vary diurnally and, in particular, that net CO₂ fluxes may be overestimated (Tang et al. 2005; Yamulki et al. 2013). Gas samples were analysed for CO₂, CH₄ and N₂O concentrations on a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) with flame ionization detector and electron capture detector fitted with a Poropack Q column operated at 60 °C with an argon carrier gas. Certified gas standards (Air Products, Crewe, UK) within the range of the samples being analysed (497, 1063, 4110 ppm CO₂, 1.07, 3.03, 10.26 ppm CH₄ and 0.41, 0.99 and 2.04 ppm N₂O)

were used to calibrate the GC. Gas fluxes (CO_2 , CH_4 and N_2O) which were calculated using the approach of Holland et al. (1999) by plotting the linear accumulation of each gas over the 45 minute enclosure period.

3.3.2.2 Climatic measures

At each sampling time micro-environmental conditions were measured along with the GHG samples at each chamber location. Soil temperatures were recorded using a Tiny Tag temperature logger with internal stab probe (Gemini Data Loggers, Chichester, UK), soil temperature was measured at a depth of 7 cm. Volumetric soil moisture was measured to a depth of 6 cm using a ML2x Theta Probe and HH2 Meter (Delta T Devices, Cambridge, UK) at three locations around each chamber from which a mean was calculated. Continuous measurements of precipitation were made from an automated WXT 520 weather station (Vaisala, Vantaa, Finland) which was installed in the grassland in block 2.

3.3.2.3 Soil sampling and processing

Soil cores (5 cm × 15 cm) were collected adjacent to (within 2 m) each chamber location on each sampling date (May 2013 – May 2014) and returned to the laboratory where they were stored at 4° C. Soil samples were used to determine the depth of the soil organic layer, soil pH, gravimetric soil moisture and available N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) (5 months only; May 2013, August 2013, November 2013, February 2014, May 2014).

To determine pH, fresh sub-samples were sieved to 2 mm to remove stones and roots and then 10 g of soil was mixed well with 25-ml of deionised water and allowed to stand for 30 minutes, before the pH of the liquid layer was recorded using a Hanna pH 210 Benchtop Meter (Hanna Instruments, Woonsocket, RI, USA). Gravimetric moisture was determined from a 10 g subsample placed in an oven at 105° C for 24 hours. Inorganic N concentration was determined by extraction with 6% KCl extraction. The extracts were analysed for $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ colourimetrically using an AQ2 discrete analyser (Seal Analytical, Southampton, UK).

3.3.2.4 Litter input and decomposition

Litter input was measured using litter interception trays (50 cm × 50 cm) (Stewart Garden, Banbury, UK) installed adjacent to each chamber from June 2013. Litter was collected from the trays monthly, bagged and returned to the laboratory where it was dried in a controlled temperature room at 30° C until a constant mass was achieved.

Litter decomposition was measured over 11 months from October 2013 to September 2014 using litterbags (Fig. 3.2E and 3.2F). The 10 cm × 10 cm litterbags were constructed from 1 mm nylon mesh (PlastOK, Birkenhead, UK) and filled with 3 g of air-dried litter which had been collected from the SRF plots in September 2013. The litterbags were sealed using a heat sealer, tagged with individual numbers and then pinned to the soil surface using metal pins in the litter layer at each chamber location. Litterbags were removed after 1, 3, 6, 9 and 11 months, returned to the laboratory where litter was carefully removed from the bags and then dried at 80° C for 24 hours to determine mass loss. A decomposition constant was derived for each plot by regression of the log of litter mass remaining against years.

3.3.2.5 Water table depth

Water table depth was measured on each sampling date using dip wells (Fig. 3.2D). Dip wells made from 1.5 m lengths of PVC pipe with 6 mm holes drilled at 5 cm intervals (from 0-1 m) to allow water in were installed using a pneumatic corer to 1 m depth in each plot at a location roughly equidistant from each of the three static chambers.

3.3.2.6 Data processing and statistical analyses

To estimate a GWP for each habitat, soil fluxes of CH₄ and N₂O were converted to CO₂ equivalents based on their GWPs of 34 and 298 respectively according to the 100-year time-frame (IPCC, 2013), and then added to the CO₂ flux. A cumulative measure for monthly GHG fluxes and the derived GWP as CO₂ equivalents were calculated

for each habitat by summing data from each plot across all sampling dates. A mean value for N₂O flux as a percentage of GWP was also calculated for each plot.

Linear mixed effects models were used to examine the differences between habitats using the nlme package. The effect of habitat on summed GHG, soil and litter decomposition variables was tested with a random effect for block. Differences between habitats were tested using post-hoc multiple comparison tests with the 'ghlt' function in the 'multcomp' package (Hothorn et al., 2008). The effect of tree species, time and their interaction on GHG fluxes was tested with a random effect for block and plot nested within block; these analyses included only tree species data (i.e. no grassland).

Drivers of GHG fluxes were explored by plotting GHG data from all sampling months against measured variables (soil temperature, soil pH, volumetric moisture, water table depth and litterfall). The relative importance of habitat and soil variables as explanatory variables for GHG fluxes was examined following Chen et al. (2015). Initial models contained fixed effects for habitat, soil temperature, soil pH, volumetric moisture, water table depth, litterfall, and interactions between habitat and these variables, and random effects for block and plot nested within block. Estimating the proportion of variance (R²) explained by the fixed, random, and residual effects was conducted using the rsquared.lme function by Jon Lefcheck (R code available at < <http://jonlefccheck.net/2013/03/13/r2-for-linear-mixed-effects-models/> >). The total R² of fixed effects was assigned to factors using the pamer.fnc function from the 'LMERConvenienceFunctions' package. The relative importance of different drivers of soil GHGs was subsequently examined under each habitat separately. The relationship between N₂O flux and rainfall at 24, 48 and 72 hours prior to GHG sampling was tested to assess rainfall lag effects.



Figure 3.2. Examples of plots and set-up at Gisburn Forest Experimental site. (A) Static chamber on a planting ridge in the Scots pine plot in Block 3, (B) Static opaque chamber in a trough of the common alder plot in Block 3, (C) Block 1 grassland with Sitka spruce plot in the background, (D) Water table dipwell in the Sitka spruce plot in Block 2; also shows lack of understory cover and dense litter cover, (E) Freshly placed litter bags in Block 3 common alder plot; also shows understory and litter cover, and (F) Litter bags in Block 2 Scots pine; also indicating grassy understory and pine needle cover. All images taken by Kim Parmar, 2013/2014.

3.4 Results

3.4.1 LUC impacts on soil GHG fluxes across tree species

There was an effect of habitat on net CO₂ flux ($F_{3,30} = 19.89$, $p < 0.001$) and cumulative fluxes were lower under all SRF species compared to the grassland. The greatest difference in net CO₂ flux following LUC was observed in the pine soils ($p < 0.001$), followed by alder soils ($p < 0.001$) then Sitka soils ($p < 0.001$). Despite this variation, there was only a significant difference in net CO₂ flux between Sitka and pine ($p = 0.015$) (Fig. 3.1A).

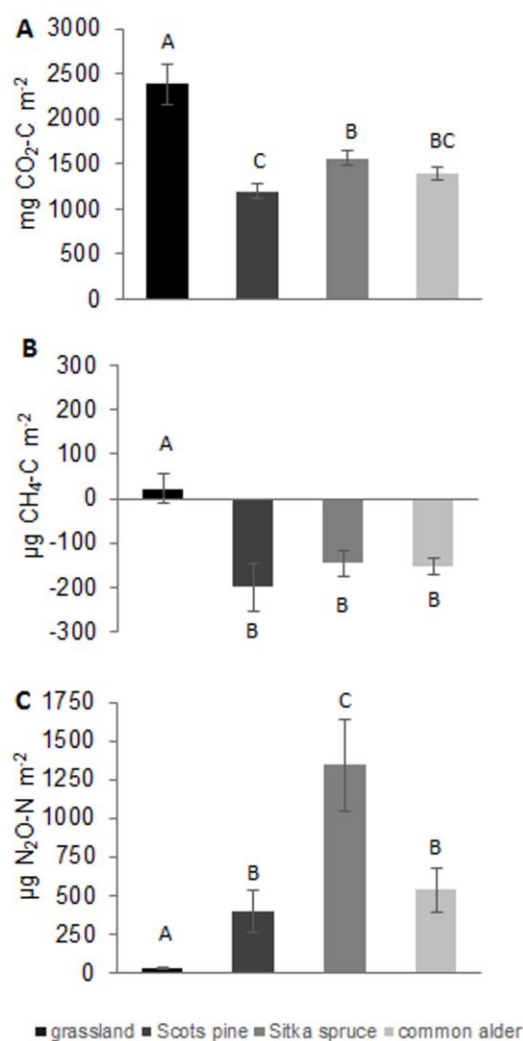


Figure 3.1. Cumulative net greenhouse gas fluxes to atmosphere for all habitats, summed from 16 monthly *in situ* gas measurements. Data are presented for individual gases in fluxes of net CO₂-C, CH₄-C and N₂O-N. Significantly different ($P < 0.05$) mean values are indicated by different letters; error bars represent standard error.

LUC from grassland to SRF resulted in the soil system becoming a small sink for CH₄ with the greatest consumption of CH₄ measured in soils under pine. Alder and Sitka soils had a similar CH₄ sink strength to each other but grassland soil was generally a minor source of CH₄ (Fig. 3.1B).

In contrast to the CH₄ and net CO₂ results, LUC to SRF resulted in the soils becoming significant sources of N₂O ($F_{3,30} = 35.06$, $p < 0.001$) with highest cumulative emissions recorded from soils under Sitka spruce. Although N₂O emissions were not as high from the common alder or Scots pine soils they were significantly higher than the grassland but significantly lower than the Sitka spruce N₂O emissions (Fig. 3.1C)

3.4.2 Differences between SRF species through time

There were differences in net CO₂ flux between SRF species ($F_{2,22} = 8.15$, $p = 0.002$) and sampling date ($F_{15,360} = 67.16$, $p < 0.001$) (Table 3.2; Figure 3.2A). Fig. 3.2A illustrates the apparent seasonal differences in net CO₂ fluxes with emissions peaking in the summer months before declining in the winter under all SRF species. The variation between species in terms of when fluxes increase and decrease across the sampling period, was supported by a significant interaction between species and sampling date ($F_{30,360} = 11.33$, $p < 0.001$; Table 3.2).

Although there was no difference between species ($F_{2,22} = 12.20$, $p = 0.398$; Table 3.2) there was an effect of sampling date on soil CH₄ fluxes ($F_{15,360} = 5.02$, $p < 0.001$; Table 3.2) illustrated in Fig. 3.2B. Despite the lack of a significant interaction between date and species ($F_{30,360} = 0.79$, $p = 0.785$; Table 3.2), there did appear to be species-related patterns. In the grassland CH₄ flux is either negative or positive depending on the time of year, with most of the consumption occurring in summer months and production in the autumn, winter and spring. The SRF soils were net sinks for CH₄ but were variable in terms of net consumption with peaks occurring in October 2013, March 2014 and July 2014 and a general decline in consumption in the winter months across all tree species.

Table 3.2. Summary statistics for the effects of tree species, sampling date and their interaction on soil GHG fluxes and net CO₂, NS = not significant, * = p ≤ 0.05, *** = p ≤ 0.001

	Tree species (T)	Sampling date (S)	T×S
CO ₂ – C	*	***	***
CH ₄ – C	NS	***	NS
N ₂ O – N	*	***	***

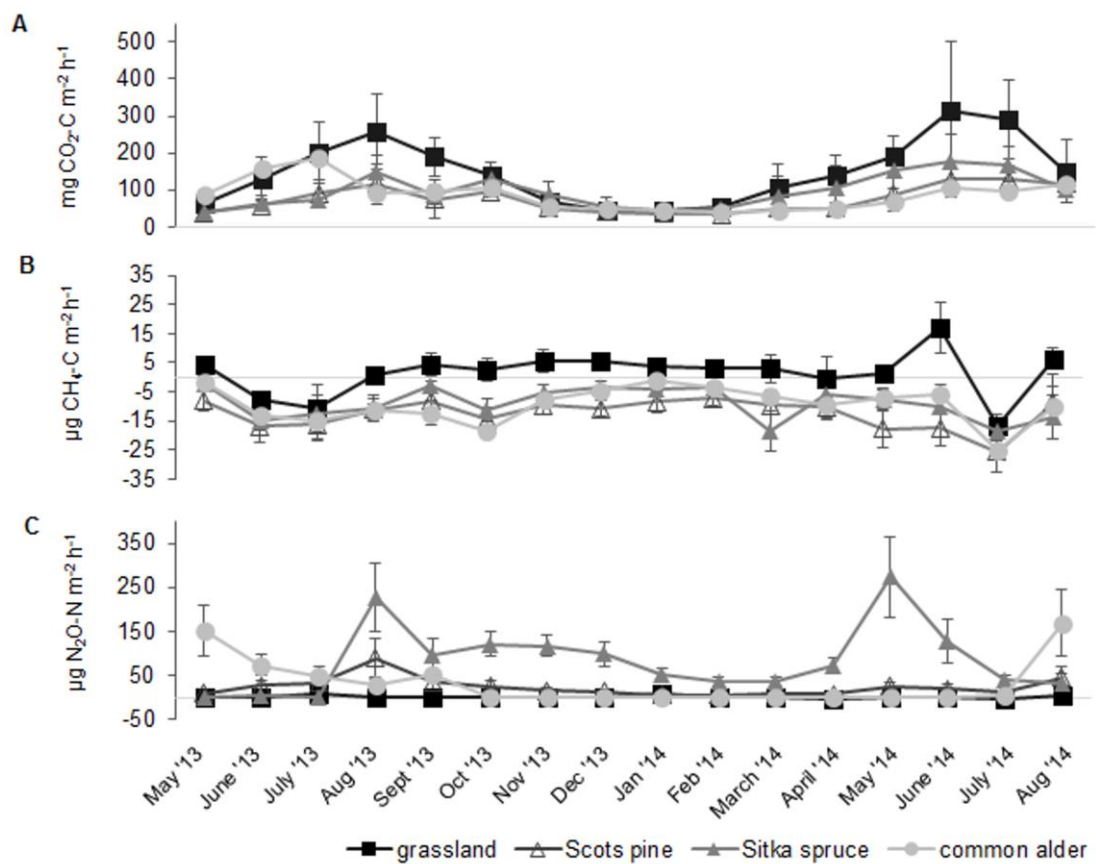


Figure 3.2. Soil GHG fluxes for all habitats over 16 months. (A) net CO₂, (B) CH₄, (C) N₂O. Error bars represent standard error; n = 9.

Soil N₂O flux was strongly affected by sampling date ($F_{15,360} = 4.16$, $p < 0.001$) as well as by species type ($F_{15,360} = 8.05$, $p = 0.002$), with emissions declining in the late autumn through to early spring (Fig. 3.2C). The interaction between species and sampling date ($F_{30,360} = 11.33$, $p < 0.001$) highlights the variation between tree species with regard to when emissions increase and decrease. N₂O emissions from common alder soils reached their highest in May 2013, and was similar to the peak in August 2014. Sitka spruce soils, which emitted the greatest amount of N₂O across the sampling period peaked in August 2013 and May 2014.

3.4.3 Litterfall and litter decomposition

Over the 12 month period in which litterfall quantity was measured cumulative values of the mass of dry litter collected from the interception trays decreased in the order of Scots pine (5481.52 g m⁻²) > common alder (4291.64 g m⁻²) > Sitka spruce (3533.88 g m⁻²) > grassland (186.44 g m⁻²). As well as species variation in litter mass input there was also temporal variation apparent (Fig. 3.3). The litterfall for Scots pine increased between June and July 2013 before reaching its maximum in August 2013 (207.84 g m⁻²), from which point litterfall started to decline fairly steadily until November 2013. The opposite occurred in common alder plots where there was minimal litterfall until September 2013 after which point litterfall increased until it peaked in November 2013 (232.24 g m⁻²) before decreasing to near-zero in December 2013. Generally, litterfall in the Sitka spruce plots was very low (2.8–106.02 g m⁻²) but consistent throughout the year, there were small peaks in input in July 2013, October 2013 and December 2013. There was little or no litterfall in the grassland plots regardless of sampling date (0 – 14.12 g m⁻²).

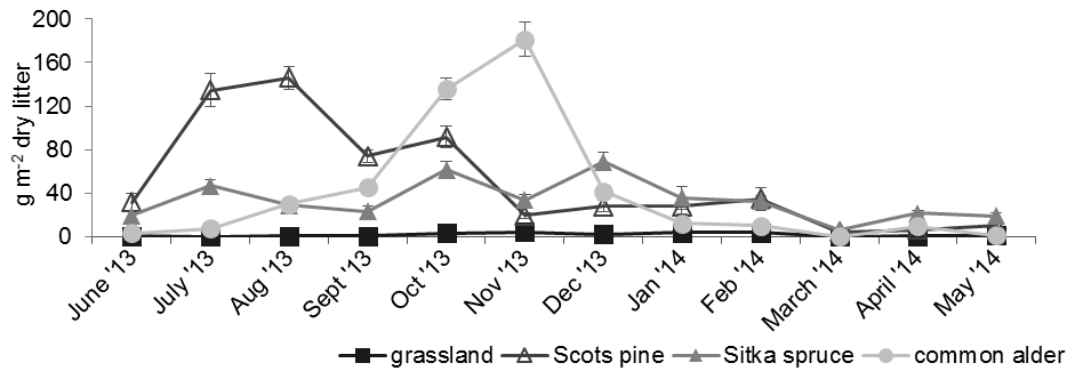


Figure 3.3. Litterfall data for all species. Data represents mean mass of litter collected from the litter interception trays and dried at 30 °C. Error bars represent standard error, n = 9.

There were differences ($p < 0.001$) between tree species in final litter mass remaining over the 337 day period for which litter bags were in-place in the field, decreasing in the order of common alder (57%) > Scots pine (64%) > Sitka spruce (74%) (Fig. 3.4A). As well as there being differences in the total mass remaining between species, the dynamics of mass loss varied between the tree species over the 11 month period. After 30 days there was no difference in mass remaining between Scots pine and Sitka spruce ($p = 0.960$) but thereafter there were generally significant differences between all species, with the exception of Scots pine and common alder after 180 days ($p = 0.092$). Annual decomposition constants (k) showed that after 11 months there was no difference between common alder and Scots pine ($p = 0.20$) with regard to litter decomposition. However, the decomposition constant of Sitka spruce was significantly lower ($p < 0.001$) than both the Scots pine and common alder litters (Fig. 3.4B).

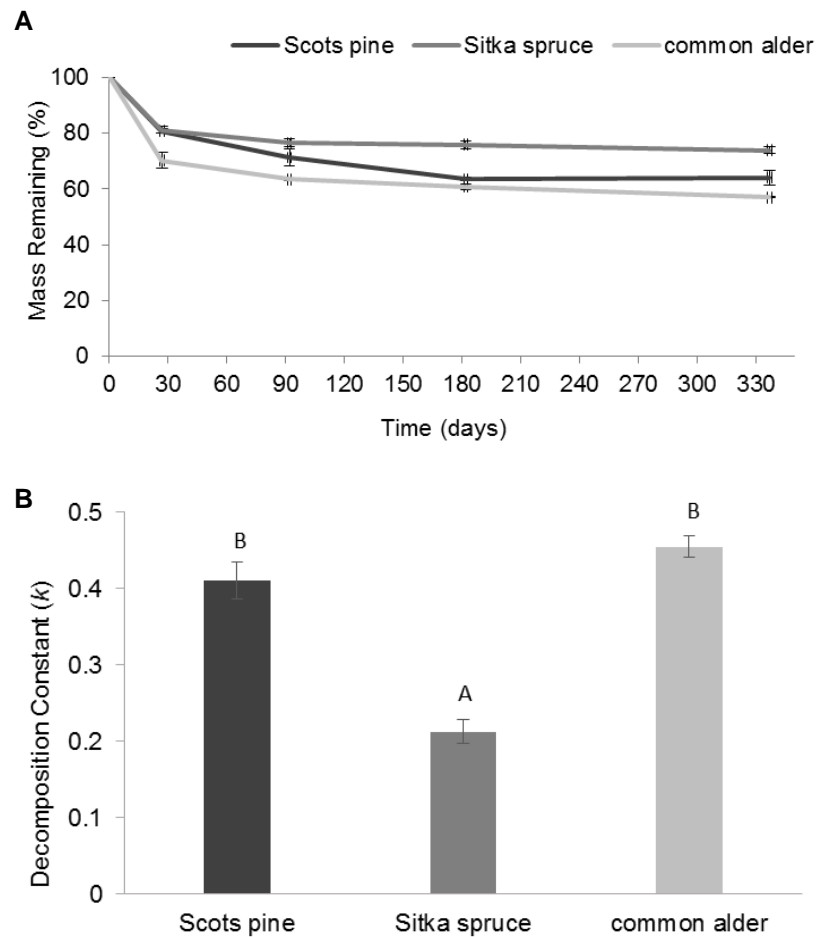


Figure 3.4. (A) Mass of litter remaining in litterbags extracted from the field for each tree species after 30, 90, 180 and 337 days. (B) Annual decomposition constants (k) for each litter species. Error bars represent standard error ($n = 9$).

3.4.4 Drivers of soil GHG fluxes under different habitats

Habitat and the interactions between habitat and measured variables were important drivers of GHGs and explained 17% of variation for net CO_2 , 14% of variation for CH_4 and 26% of variation for N_2O (Table 3.3). For net CO_2 and N_2O fluxes the most important driving interactions were between habitat and soil temperature and habitat and water table depth. Whereas, habitat and soil moisture, and habitat and litterfall were the greatest driving interactions for CH_4 flux (Table 3.3). The highest unexplained variation (residuals) applied to N_2O flux, this was similar for CH_4 but

there was a lot less unexplained variation in the net CO₂ flux due to a large percentage being explained by soil temperature (Table 3.3).

Table 3.3. The percentage of variation (as determined by linear mixed models) in soil GHG fluxes and net CO₂ flux as explained by habitat, measured variables and their interactions at Gisburn Forest experimental site.

	CO ₂		CH ₄		N ₂ O	
	%	P	%	P	%	P
<u>FIXED EFFECTS</u>						
Habitat	6.14	<0.001	5.05	<0.001	8.34	<0.001
Soil Temp.	42.82	<0.001	5.12	<0.001	3.83	<0.001
pH	1.13	<0.001	0.66	0.106	0.11	0.481
Soil moisture	0.12	0.266	3.12	<0.001	1.07	0.026
Water table depth	0.00	0.973	2.06	0.005	0.75	0.062
Litterfall	0.62	0.012	0.36	0.230	0.13	0.440
Habitat × Soil temp.	6.17	<0.001	1.55	0.106	3.50	0.001
Habitat × pH	0.76	0.054	1.85	0.064	0.46	0.540
Habitat × Soil moisture	0.63	0.095	2.44	0.022	1.61	0.059
Habitat × Water table	2.49	<0.001	0.95	0.287	10.93	<0.001
Habitat × Litterfall	1.17	0.008	2.64	0.016	1.41	0.088
<u>RANDOM EFFECTS</u>						
Block/Plot	9.09		20.21		11.41	
<u>RESIDUALS</u>						
	28.83		53.98		56.43	

Focusing on habitat-specific drivers of GHGs it is evident that the influence of certain variables on fluxes is species dependent (Fig. 3.5). In the grassland habitat, most of the variation in net CO₂ flux was explained by soil temperature and sampling location (block & plot), soil pH and litterfall also contributed in small proportions (Fig. 3.5A). For CH₄ in grassland, variation was mostly generally explained by sampling location, there was also some influence of soil moisture, water table depth, pH, soil temperature and litterfall (Fig. 3.5B). There was no effect of sampling location on N₂O fluxes in grassland where N₂O was mostly driven by moisture, both as percentage soil moisture and depth to water table, and by soil pH (Fig. 3.5C).

Soil temperature and spatial effects (block & plot) were also the greatest drivers of net CO₂ flux in the Scots pine habitat. Of the other measured variables, only water table depth appeared to have any influence on net CO₂ fluxes in Scots pine (Fig. 3.5A). The spatial effects of block and plot were a key driver of CH₄ in the Scots pine habitat, explaining over half of the total variation in flux, and there were small but equal influences of litterfall, soil temperature, water table depth and soil moisture on Scots pine CH₄ fluxes (Fig. 3.5B). Soil N₂O fluxes were mostly driven by the spatial effects of block and plot and by soil temperature, soil moisture also had a small influence on N₂O flux (Fig. 3.5C).

In the Sitka spruce soils spatial variation and soil temperature affected net CO₂ equally and there was also a small effect of water table depth (Fig. 3.5A). CH₄ fluxes in the Sitka spruce plots were mostly driven by spatial variation but percentage soil moisture also had an effect (Fig. 3.5B). The spatial effects of block and plot and depth to water table were the most important drivers of N₂O flux from Sitka spruce soils, but there were also small effects of soil temperature and soil moisture (Fig. 3.5C).

Soil temperature was the greatest driver of net CO₂ fluxes in common alder soils (Fig. 3.5A) and only small amounts of variation were explained by water table depth and soil moisture. In contrast to the other habitats there was very little variation in soil GHGs explained by the spatial effects of block and plot (Fig. 3.5). Soil temperature and pH were stronger drivers of CH₄ fluxes in common alder soils compared to other

habitats and there was also a small effect of litterfall (Fig. 3.5B). Alder N₂O fluxes were driven by soil temperature, water table depth (Fig. 3.5C).

The variables measured did not explain all of the variation in soil GHG fluxes and for each habitat unexplained variation was captured in the residuals. For net CO₂ flux there was more than double the variation (71%) left unexplained in the Sitka spruce soils compared to the others. Common alder soils had the greatest amount of unexplained variation (75%) for CH₄ fluxes and Scots pine had far less unexplained variation in N₂O fluxes compared to grassland, common alder and Sitka spruce which were all over 60%.

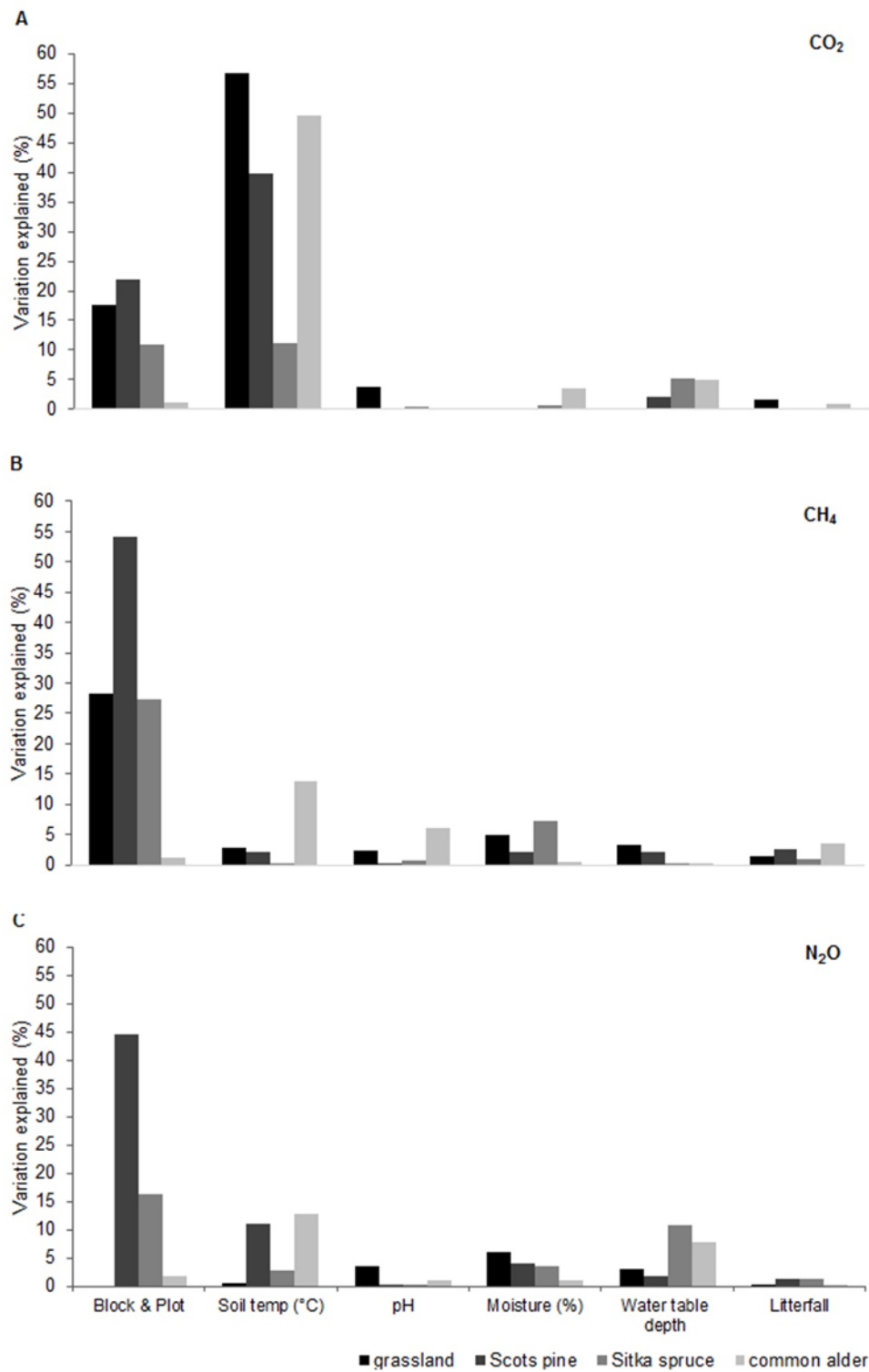


Figure 3.5. Percentage variation (as determined by linear mixed models) in soil GHG fluxes as explained by measured variables in each habitat. (A) net CO₂ flux, (B) CH₄ flux, and (C) N₂O flux. Block & Plot were random factors in the model. Values for residuals have been omitted from these plots.

No effect of rainfall (mm) on N₂O flux was found considering lag times of 24 hr (P = 0.984), 48 hr (P = 0.858) and 72 hr (P = 0.951) prior to GHG sampling. N₂O fluxes from all species showed consistent rates irrespective of rainfall events in the 72 hours preceding measurements. (Appendix A.2.).

3.5 Discussion

3.5.1 How does LUC from grassland to SRF impact GHG emissions?

Land use change is the second largest source of anthropogenic GHG emissions, mainly due its effect on the cycling and storage of soil C and the soil-atmosphere exchange of CO₂, CH₄ and N₂O (Rounsevell & Reay, 2009; Arevalo et al., 2011; Don et al., 2011). Increased bioenergy production, as an alternative energy source to fossil fuels, may result in considerable LUC to SRF (amongst other bioenergy crops). As part of the rationale for growing bioenergy is that they can contribute to mitigating GHG emissions (Osborne & Jones, 2012) there is an urgent need to quantify the effects this could have on the GHG balance.

This study set out to examine the effects of LUC to SRF on soil GHG and net CO₂ fluxes at Gisburn experimental forest site in Lancashire, north-west England. It was expected that LUC to SRF would result in a reduction in CH₄ emissions and an increase soil N₂O emissions. Decreased CH₄ emissions were predicted due to the existence of conditions favourable for CH₄ consuming methanotrophic bacteria (Christiansen & Gundersen, 2011), increased soil diffusion of oxygen and lower water table depths (Ball et al. 2007). Increased N₂O emissions were expected due to higher levels of available N (Liu & Greaver, 2009), increased soil acidity (Weslien et al., 2009), conditions favourable for N₂O producing microbial activity (Ambus et al., 2006), increased soil aeration due to lower water table and the presence of thick soil organic layers (Ball et al., 2007). Decreased CH₄ emissions and increased N₂O emissions were found in this study. All three tree species transitions resulted in a significant reduction in CH₄ fluxes compared to the ungrazed rough grassland, and there was a significant increase in soil N₂O emissions with species related differences in magnitude.

Decreased CO₂ emissions may be expected due to tree related increased recalcitrant C inputs above and below ground and slower litter decomposition rates (Morison et al., 2012). In this study, using the method described, a decrease in net CO₂ fluxes was

measured following LUC from ungrazed rough grassland to SRF. It was not possible, however, to separate the components of respiration and a large proportion of the net CO₂ flux measured may be due to dark respiration of grass. Grass was abundant in the alder and Scots pine but not in the Sitka spruce plots, and therefore care must be taken in drawing conclusions from the CO₂ flux results.

3.5.2 Are there differences in GHG fluxes between tree species following LUC and do they change over time?

It was predicted that there would be differences in GHG emissions following LUC between tree species due to known differences between broadleaved and coniferous species and as a result of N-fixation by common alder. While this was found to be true for net CO₂ and N₂O, there were no differences in CH₄ fluxes between tree species. The effect of sampling date on CO₂ and N₂O fluxes is well known as the microbial production of these gases is sensitive to temperature and moisture conditions which tend to be more favourable in the summer months (Davidson et al., 2002; Smith et al., 2003; Trumbore, 2006).

Of all natural systems, temperate forests have the highest CH₄ uptake potential (Skiba et al., 2009; Rowlings et al., 2012) with a mean rate of 3.6 kg CH₄-C ha⁻¹ y⁻¹ (Dalal & Allen, 2008) and an upper limit of 8.9 kg CH₄-C ha⁻¹ y⁻¹ (Bowden et al., 2000). Higher consumption rates are usually recorded in broadleaved compared to coniferous forests (Jang et al., 2006; Skiba et al., 2009). This is likely to be because of a reduction in diffusion capacity under conifers compared to broadleaves due to the presence of a thick surface organic layer, and because of the tree species influence on the abundance and composition of the methanotrophic microbial community (Butterbach-Bahl & Papen, 2002; Boriken et al., 2006). In our study, net cumulative CH₄ consumption was found under all tree species, this was in contrast to net production in the grassland soils (0.13 kg CH₄-C ha⁻¹ y⁻¹). However, the mean CH₄ uptake rates at Gisburn Forest were below the global mean for temperate forests. This is not unusual, a recent study by Barrena et al. (2013) measuring soil GHG fluxes from forest soils in the Basque Country also found lower than average annual CH₄ uptake in mature

beech ($1.26 \text{ kg CH}_4\text{-C ha}^{-1} \text{ y}^{-1}$), mature pine ($0.59 \text{ kg CH}_4\text{-C ha}^{-1} \text{ y}^{-1}$) and Douglas fir ($0.23 \text{ kg CH}_4\text{-C ha}^{-1} \text{ y}^{-1}$) soils. The authors attributed this to a lack of available mineral N due to the study sites being in an area of very low N deposition ($5\text{-}7 \text{ kg N ha}^{-1} \text{ y}^{-1}$) which is thought to be a pre-requisite to induce CH_4 consumption (Bodelier & Laanbroek, 2004). As well as below average CH_4 consumption rates, we also failed to find higher uptake in the broadleaved alder soils compared to the coniferous pine or Sitka soils, and in fact Sitka and alder had almost the same uptake rates. This is likely due to alder being an N-fixer which can lead to CH_4 uptake in soil being suppressed by high levels of available N (Butterbach-Bahl et al., 1998; Reay & Nedwell, 2004). Nitrate levels in the alder soils in this study were 62% higher than in the pine, and 90% higher than in the Sitka soils. In contrast to the Basque Country location of the study by Barrena et al. (2013), the overall low CH_4 uptake rates across all species may be a result of Gisburn Forest being in an area of exceptionally high atmospheric N deposition ($44.38 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for deposition years 2010-2012) (APIS, 2015).

The measured mean soil N_2O fluxes in this study were within the range reported by Dalal & Allen (2008) for temperate forests of $7.04 - 7744 \text{ g N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$. However, the means for all tree species (Sitka spruce, 7356.2, common alder, 2934.9, Scots pine, 2152.7 $\text{g N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$) were considerably higher than Dalal & Allen's (2008) reported mean of $1513.6 \text{ g N}_2\text{O-N ha}^{-1} \text{ y}^{-1}$ indicating that the Gisburn Forest site soils are a relatively large source of N_2O compared to other temperate forests but that there are key species related differences effecting the magnitude of emissions. Although variable results exist within the literature with regard to tree species type effects on soil N_2O emissions, many studies have found higher N_2O emissions from broadleaved compared to coniferous soils mainly due to differences in litter quality and soil moisture (Ambus et al., 2006; Pilegaard et al., 2006). Therefore, it was surprising that N_2O emissions from common alder soils were so much lower than from Sitka spruce soils. As well as being broadleaved, common alder is also an N-fixing species, and through its symbiotic relationship with dinitrogen (N_2) fixing bacteria from the *Frankia* group (Rytter et al., 1989) it can have large amounts of

available N in the soil system (Reay et al., 2005). As reported above we found higher levels of nitrate in the common alder soils compared to the others, however ammonium values were lowest in Alder soils (Sitka spruce, 23.24, Scots pine, 20.29, common alder, 17.75 mg NH₄-N kg⁻¹). The availability of N is one of the key regulators of N₂O production as NH₄⁺ and NO₃⁻ are the precursors for nitrification and denitrification, respectively (Pihlatie et al., 2007). It is known that atmospheric N deposition is higher in coniferous stands compared to broadleaved stands (Gunderson et al., 2009) as a result of conifers having a higher leaf area index and longer foliage longevity (De Schrijver et al., 2007). This could have led to greater N₂O emissions from Sitka spruce soils compared to common alder, but does not explain the lower fluxes from Scots pine soils.

Both nitrification and denitrification are moisture sensitive due to the effects moisture has on oxygen availability (Barnard et al., 2005; Gillam et al., 2008), when moisture content is high N₂O production from incomplete denitrification dominates (Bateman & Baggs, 2005) whereas nitrification peaks at intermediate moisture (< 60% water holding capacity) (Case et al., 2012). At Gisburn Forest soil volumetric moisture was higher in the common alder plots (81%), compared to the Sitka spruce plots (57%), with Scots pine intermediate (78%) but the depth to water table was higher in the Sitka spruce plots (24 cm) compared to common alder and Scots pine, which had the same mean depth (31 cm). This suggests that the low rates of N₂O production in the common alder plots at Gisburn are due to conditions generally not being favourable (too wet) for incomplete denitrification despite the higher levels of NO₃ availability. The explanation for the higher N₂O emissions in the Sitka spruce plots might be due to a combination of processes for which favourable conditions exist at different times. For instance, N₂O emissions were highest in the Sitka spruce plots when water table depth was intermediate (20-30 cm) and negligible when the water table was low (below 40 cm), suggesting that incomplete denitrification was occurring. This was in agreement with the findings of Jungkunst et al. (2004) who measured lower N₂O emissions at a water table depth of 65-75 cm but higher emissions when the water

table was intermediate at 15-35 cm. Soil volumetric moisture conditions in the Sitka spruce habitat (mean 57%) might also be optimum for N₂O production at certain times, a study by Ball et al. (2007) measuring GHG fluxes under Sitka Spruce in Northumberland identified 40-50% vmc as being optimum for N₂O production. It is also possible that N₂O is being produced in the deep soil organic layer (mean 4.35 cm) that exists in the Sitka spruce plots due to the abundance of ectomycorrhizal fungi. All of these processes could be supported by high available N due to increased N deposition as a result of a dense canopy and lack of understory vegetation in the Sitka spruce plots. A study carried out by Prendergast-Miller et al. (2011) using Sitka spruce root tips demonstrated for the first time that ectomycorrhizal fungi can produce N₂O from nitrate reduction in soils receiving high rates of N deposition.

Soil CO₂ fluxes in forests can be very variable, largely due to site variation which is a product of the interactions between climate, soil type, topography, soil microbial community and species type, all of which directly or indirectly affect CO₂ fluxes (Raich & Tufekcioglu, 2000; Saiz et al., 2006; Schaulfler et al., 2010; Vesterdal et al., 2012; Barrena et al., 2013). By carrying out a study at a single site we controlled for variation in CO₂ fluxes driven by soil type and climate, thus allowing us to better focus on species related effects, an approach recommended by Vesterdal et al. (2012). We were, however, unable to account for the contribution that dark respiration of ground vegetation to soil CO₂ fluxes; ground vegetation cover was complete in the grassland (100%) and the common alder plots (100%), and high in the Scots pine (~70%).

While species related differences were observed, we did not find support for the hypothesis that alder would have the greatest net CO₂ emissions. This was anticipated, as alder is an N-fixing species with a higher predicted rate of nutrient turnover and therefore higher subsequent CO₂ flux compared to non N-fixing species (Kim et al., 2012). Alder litter also had the highest decomposition rate in this study, with only 57% remaining after 11 months compared to 64% in pine and 74% in Sitka. It was also unexpected due other research that coniferous forests have lower rates of

CO₂ flux than adjacent broadleaved forests growing on the same soil type (Raich & Tufekcioglu, 2000). However, a meta-analysis by Subke et al. (2006) found no significant difference in soil respiration between temperate coniferous and broadleaved species, a finding that was shared by a recent study by Vesterdal et al. (2013). Similarly to our findings, Barrena et al. (2013) observed higher annual CO₂ emissions from pine soils compared to beech and Douglas fir soils, and attributed these differences to the presence of a high percentage of ground cover vegetation in the pine (80 - 90%) compared to the beech (< 20%) and fir (< 20%). This cannot be the case at Gisburn Forest as there is little ground cover vegetation in the Sitka plots, compared with almost complete ground vegetation cover in the alder and pine plots. Instead, the reason for higher CO₂ flux from the Sitka soils might be related to the presence of ectomycorrhizal fungi that are often most abundant in the deep litter layers of acidic forests (Genney et al., 2006). A study by Moyana et al. (2008) looking at soil respiration in relation to photosynthetic activity in Germany found that mycorrhizal mycelium respiration was 8% in a Norway spruce forest compared to 3% in a beech forest. Ectomycorrhizal fungi will also be present in the alder and pine soils, but development is promoted where litter inputs are more recalcitrant, organic layers are deep and where there is a lack of understory growth for mycorrhizal development (Prendergast-Miller et al., 2011).

3.5.3 Which soil and environmental variables are the key drivers of GHG fluxes under different tree species?

Soil greenhouse gas fluxes in forests are controlled by a range of natural and anthropogenic factors and interactions between these factors including soil moisture, water table depth, soil aeration, pH, soil temperature, soil type, land management, species selection and stand age (Morison et al., 2012). In particular, soil temperature and moisture influence soil-atmosphere GHG exchange due to their effects on soil microorganisms and roots (Smith et al., 2003). Rates of these processes responsible for GHG exchange generally increase exponentially with temperature as long as other factors are not limiting (Meixner & Yang, 2006). Our findings were in agreement with

this where we found a significant proportion of the variation in each GHG was attributed to soil temperature, and it was in fact the most important driver of net CO₂ fluxes across all habitats. Soil moisture is an important driver of soil GHGs as it is an important substrate for soil microorganisms (Meixner & Yang, 2006), and influences gas diffusivity (Smith et al., 2003). Generally, CO₂ emissions increase exponentially with increasing soil moisture, however emissions may be reduced under very wet or very dry conditions (Schaufler et al., 2010). Under anaerobic conditions CH₄ is produced and under aerobic conditions CH₄ is consumed (McNamara et al., 2008), whereas N₂O can be produced under both aerobic and anaerobic conditions. Despite the large range of soil moisture conditions (10 – 100 % vmc) measured over the 16 month gas sampling period at Gisburn Forest we found no relationship between soil moisture and net CO₂ fluxes. Soil moisture did explain a significant percentage of the variability in soil CH₄ and N₂O fluxes overall. We also found that habitat was an important driver of all soil GHG fluxes, as were the interactions between habitat and other measured variables suggesting that different species have different GHG drivers even in the same soil type.

Depth to water table and its effects on soil aeration can have a significant impact of soil GHG fluxes, particularly in organic soils. Water table drawdown increases soil aeration in the upper soil layer which can increase microbial soil organic matter decomposition and subsequent N mineralisation leading to a rise in CO₂ and N₂O production (Freeman et al., 1996; Minkinen et al., 2002; Martikainen et al., 1993). Increased aeration has the opposite effect on CH₄ production which favours anaerobic conditions (McNamara et al., 2008). In this study we found that water table depth had a significant effect on CH₄ fluxes regardless of habitats, with increased CH₄ consumption measured under all tree species where soils were generally drier than in the grassland which was a net CH₄ producer. This matches the results from a study by McNamara et al. (2008) examining the influence of afforestation and tree species on soil CH₄ fluxes at Gisburn Forest, where positive CH₄ fluxes were measured from the grassland soils and negative fluxes from Norway spruce, Sessile oak, common

alder and Scots pine soils and attributed to water table depth and soil moisture content. We also found that the interaction between habitat and water table depth was an important driver of net CO₂ and N₂O fluxes. It was clear to see from the habitat specific analysis that this influence of water table was in the Sitka spruce and common alder soils and might therefore be linked to N availability via N-fixation and N deposition, and the physical and biological conditions favourable for CO₂ and N₂O production as discussed previously. A study by Ball et al. (2007) found higher N₂O emissions in soils from 30 year old Sitka spruce stands compared to 20 year old stands due to better aeration of older soils as a result of deeper water table which facilitated the release of substrate required for N₂O production.

The generally high percentage of variation explained by the random effect for block and plot, particularly for CH₄ and N₂O fluxes under Scots pine and Sitka spruce, suggest that in these habitats sampling location may also be a driver of soil GHG fluxes. These block and plot factors will encompass variation attributable to microtopography created during forest planting. It is thought that land management, in terms of ground preparation prior to planting and subsequent creation of microtopographies (ridges, furrows (troughs), undisturbed flats) can have an effect on both initial and long-term soil GHG emissions and C storage. Before carrying out their work on stand related effects on soil respiration in a Sitka spruce chronosequence, Saiz et al. (2006) did a preliminary study testing the effects of microtopography (furrows, ridges, flats) and chamber position on soil CO₂ flux. They found that there were differences in respiration rates depending on chamber location, with the highest rates measured from furrows. Whether gas sampling chambers were situated on flats, furrows or ridges in Gisburn Forest could be influencing GHG fluxes due to microtopographical differences in depth to water table, organic layer depth, location of roots, litterfall and decomposition, and soil temperature.

3.6 Conclusion

In this single site study LUC from rough ungrazed grassland to SRF resulted in, increased CH₄ uptake, and increased soil N₂O emissions. There were also significant differences between tree species with higher net CO₂ and N₂O emissions measured from Sitka spruce soils compared to common alder and Scots pine. Net CO₂ fluxes were driven largely by soil temperature. Random spatial effects explained a large proportion of the variation in soil CH₄ flux. Whereas, N₂O flux was driven by habitat and the interaction between habitat and water table depth. The interactive effects between tree species and season on N₂O emissions also suggest that SRF species must be considered carefully for potential GHG mitigation. Future work should focus on whether these patterns are consistent across other soil types and geographical zones.

Chapter 4: Effects of tree species, water table and microtopography on soil GHG fluxes

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Declaration of contribution

This laboratory based study was planned and carried out by Kim Parmar. Mesocosm extraction was carried out by Kim Parmar with assistance from Dr Aidan Keith and Andrew Fitton. All sampling, and analyses were conducted by Kim Parmar, with the exception of the $\text{NH}_4^+/\text{NO}_3^-$ measurements which were made by Benjamin Jackson. Data were analysed with help from Dr Aidan Keith and the chapter was written by Kim Parmar and reviewed and commented on by Dr Aidan Keith, Dr Niall McNamara and Dr Saran Sohi.

4.1 Abstract

LUC from grassland to forest has been shown to change soil C and GHG fluxes but generally with high variability. This variability may be due to differences between tree species and the effects they can have on soil hydrological properties. Management of forests (e.g. site preparation) can also impact the physical structure of the soil and this may modify the impacts of trees on soil C and GHG fluxes.

Soil mesocosms were collected from under different tree species (Scots pine, Sitka spruce and common alder) and grassland at the Gisburn Forest Experiment. The mesocosms were taken from different microtopographies (undisturbed flats, ridges, troughs) under each tree species, which occurred as a result of pre-planting site preparation. These were subjected to high water table (3 cm) and low water table (27 cm) treatments, and GHGs were measured over 134 days. A nitrogen (N) addition experiment was also performed after 169 days to determine whether soils were N limited under different tree species and grassland.

Water table impacted fluxes of N_2O and CH_4 but not CO_2 , with higher N_2O at low water table and higher CH_4 at high water table. There was an interactive effect of tree species and water table on N_2O emissions, with high water table decreasing N_2O in common alder and Scots pine soils, but not Sitka spruce. The effect of microtopography on N_2O flux appeared to be influenced by water table, but varied depending on SRF species, though this was not significant. Tree species had an effect on CO_2 emissions, and highest rates were measured from Sitka spruce soils under both water tables. Overall, water table was the only significant driver of soil CH_4 fluxes. Comparing the tree species effects to the grassland reference, CO_2 emissions were lower under common alder and Scots pine but higher from Sitka spruce, net emissions of CH_4 were measured from grassland irrespective of water table and N_2O fluxes were negligible from grasslands.

This study demonstrates that water table depth can modify the pattern of soil-atmosphere GHG exchange but this is also dependent on tree species. Therefore, consideration should be given to the impact that temporal fluctuations in water table will have on the magnitude and direction of soil GHG fluxes. Furthermore, these findings suggest that in forest sites GHGs need to be measured across representative microtopographies, in order for more accurate calculation of GHG budgets.

4.2 Introduction

Short Rotation Forestry (SRF) for bioenergy could deliver greater volumes of biomass from the same land area as alternative bioenergy crops such as Short Rotation Coppice (SRC) (McKay, 2011). Therefore, SRF is being seriously considered as a potential bioenergy crop to meet biomass demand and renewable energy targets (Leslie et al., 2012). As current experience of SRF in the UK is limited there is an urgent need to evaluate its viability as a fossil fuel alternative, and increased knowledge from systematic research is required to ensure that commercial SRF offers multiple environmental benefits, including GHG mitigation and C sequestration.

Forest ecosystems can store carbon (C) in the long term and, because of this, afforestation is viewed as a means to mitigate rising CO₂ concentrations in the atmosphere (Christiansen & Gundersen, 2011; Vesterdal et al., 2012). Afforestation also affects the soil-atmosphere exchange of other important greenhouse gases (GHGs) such as methane (CH₄) and nitrous oxide (N₂O), due to tree-related alteration of soil and micro-climatic conditions (Peichl et al., 2014). Since the global warming potential (GWP) of CH₄ and N₂O is greater than CO₂ (34 and 298 times, respectively) on a molar mass basis over 100 years (IPCC, 2013), increases in emissions of these GHGs could offset any C savings made as result of planting SRF (Zenone et al., 2015). Temperate forest soils have the strongest CH₄ sink potential of all natural systems with soil uptake rates up to 8.9 kg CH₄-C ha⁻¹ y⁻¹ and a mean of 3.6 kg CH₄-C ha⁻¹ y⁻¹ (Bowden et al., 2000; Dalal and Allen, 2008). However, temperate forests may also be significant sources of soil N₂O emissions (Kesik et al., 2005; Zhang et al., 2008) with estimated emission rates of 0.01-8.07 kg N₂O-N ha⁻¹ y⁻¹ (Dalal & Allen, 2008). Therefore, it is also important to consider the soil-atmosphere exchange of these three important GHGs when estimating the mitigation potential of a forest system. This mitigation potential may be further modified by tree species, for example, some studies have found that broadleaved forest soils can emit more N₂O than coniferous forest soils (Butterbach-Bahl et al., 2002; Pilegaard et al., 2006; Strange et al., 2013). A study by Barrena et al. (2013), however, measured higher N₂O emissions from coniferous soils but found there were large differences between species, with 17 times higher emissions from Douglas fir soils compared to radiata pine soils. These variable results highlight the need to evaluate species-specific effects on soil GHG emissions in order to make more informed decisions on SRF species selection in the future.

Tree species differences in root development, litter quantity and quality, soil microbial community composition, canopy shading and rainfall interception can lead to changes in soil physical, biogeochemical and hydrological properties, all of which can affect soil GHG exchange with the atmosphere (Smith et al., 2003; Borken & Beese, 2005; Ball et al., 2007; Christiansen & Gundersen, 2011; Peichl et al., 2014). For example, it is reported that nutrient turnover rates are higher in soils under broadleaved species compared to under coniferous species (Ambus & Zechmeister-Boltenstern, 2007) due to broadleaved litter having more labile C available which is easily decomposed by microbes. Furthermore, species such as alder which is a nitrogen-fixing species can affect soil nutrient status by depositing litter high in N concentration (Wedderburn & Carter, 1999), whilst soils under coniferous species such as pine and spruce generally have higher C:N ratios and lower pH than broadleaved soils (Menyailo et al., 2002). Soil N availability is also greatly influenced by rates of N deposition which vary as result of location, e.g. temperate forests in NW Europe that experience increased N deposition from air pollution (Butterbach-Bahl et al., 2002), and species type, with higher rates of N deposition usually recorded in coniferous soils compared to broadleaved soils (De Schrijver et al., 2007; Gundersen et al., 2009; Rothe et al., 2002).

The direction and magnitude of soil-atmosphere exchange of these GHGs is mainly controlled by the cycling of soil C and N. These biogeochemical cycles are in turn controlled by soil temperature and moisture due to their effects on microbial activity (Davidson et al., 1998; Bardgett, 2005), water table depth due to its effect on the oxic/anoxic boundary and soil aeration (Ball et al., 2007). Tree species type can also influence C and N cycling due to their effects on the quantity and quality of available organic substrate and soil physical and biological properties (Gleixner et al., 2005; Vesterdal et al., 2012; Prescott & Grayston, 2013). In addition, N deposition (Reay et al., 2005; Christiansen & Gundersen, 2011; Barrena et al., 2013), and management practices such as ground preparation pre-planting, and harvesting techniques (Saiz et al., 2006; McKay, 2011) impact soil C and N, and soil-atmosphere GHG exchange.

Soil water content is important as it aids substrate supply to microorganisms (Schindlbacher et al., 2004) and influences gas diffusivity (Smith et al., 2003). Rates of soil chemical and biological processes generally increase exponentially with temperature, as long as factors such as moisture are not limiting (Meixner & Yang, 2006). Depth to water table and its effect on soil

aeration is linked to soil GHG fluxes (Ball et al., 2007). Water table drawdown increases soil aeration which can lead to increased microbial soil organic matter decomposition and N mineralisation, resulting in a rise in CO₂ and N₂O production (Martikainen et al., 1993; Freeman et al., 1996; Minkkinen et al., 2002). However, increased aeration has the opposite effect on CH₄ production which favours anaerobic conditions (McNamara et al., 2008). Trees are known to use more water than shorter vegetation types (Nisbet, 2005), and although no studies have yet measured water usage in the context of SRF it is predicted that SRF plantations will exceed those of conventional forests due to faster growth rates (McKay, 2011). Coniferous SRF species are likely to have higher water usage than broadleaved species and this could further impact water table dynamics (McKay, 2011). Interactions between tree species and water table could influence the direction and magnitude of soil GHG fluxes and, therefore, it is important to consider water table depth when collecting GHG measurements.

Forest management can significantly impact soil GHG emissions, in part due to soil disturbance during extensive ground preparation. Some ground preparation techniques such as ploughing and overturning soil, to create planting ridges and improve drainage result in local microtopography being created (McNamara et al., 2008). The effect of microtopography and the interaction between microtopography and tree species on GHG exchange is uncertain. Saiz et al. (2006) tested the effects of microtopography (furrows (troughs), ridges, flats) on soil CO₂ flux in a Sitka spruce chronosequence in central Ireland and found the highest respiration rates from furrows, and linked this to the presence of thicker soil organic layers in furrows compared to flats or ridges. In contrast, Ball et al. (2007) measured higher mean CO₂ fluxes from ridges in a Sitka spruce chronosequence in Northumberland and attributed this to water table depths being lower in ridges compared to ditch sides (flats) or ditches (furrows/troughs). Whether or not the interactions between water table depth and microtopography are consistent under different tree species could have an impact on soil GHG fluxes. In order to get a representative sample of field-scale GHG fluxes in SRF, multiple samples should be taken from flats, troughs and ridges.

An earlier field study at the Gisburn Forest Experimental site (Chapter 3) examined the effects of land use change to SRF on soil-atmosphere GHG exchange. This work showed that soil temperature, depth to water table, tree species, spatial variation and interactions between these variables all explained variation in soil fluxes of CO₂, CH₄ and N₂O. It is challenging to

determine the relative influence of driving factors and their interactions in the field because soil variables, such as soil temperature and soil moisture, often co-vary through time (Fang & Moncrieff, 2001; Schaufler et al., 2010). Different tree species are also likely to have varying water demands throughout the year depending on the species growth pattern. Other variables such as N deposition, litterfall and soil N availability also vary temporally (Davidson et al., 2000; Pilegaard et al., 2006). For CO₂ fluxes there is the added complication which arises from the contribution of autotrophic respiration in field studies, this can account for up to 50 % of total CO₂ flux (Högberg et al., 2001; Bahn et al., 2006; Byrne & Kiely, 2006). In order to tease apart measures which co-vary temporally and drive GHG fluxes in field studies, controlled core (mesocosm) laboratory experiments are often used (Fang & Moncrieff, 2001; Schaufler et al., 2010; Gabriel & Kellman, 2014).

Spatial variability in soil GHG fluxes is known to be large, even in homogenous stands of tree species (Raich et al., 1990). It may be affected by root distribution (Saiz et al., 2006), the mass of litter accumulation and quality and quantity of soil C pools (Klopatek, 2002; Fang et al., 1998), and organic layer thickness (Saiz et al., 2006). These physical differences may interact with the depth to water table to modify GHG fluxes (Ball et al., 2007). Consequently, microtopography and its interactions with these variables may be particularly important (Ball et al., 2007; Fang et al., 1998; Nungesser, 2003; Saiz et al., 2006).

In order to better understand the driving effects of, and interactions between, water table, microtopography and tree species on soil GHG emissions observed in the field (Chapter 3), a laboratory controlled intact core experiment was carried out. This study examined the effects of interactions between tree species, water table and microtopography on soil GHG emissions from soils under 23 year old stands of *Picea sitchensis* (Sitka spruce) (first rotation), *Pinus sylvestris* (Scots pine) (second rotation), *Alnus glutinosa* (common Alder) (second rotation) and compare these with long-term rough grassland. This study is the first to examine these effects in a SRF bioenergy context and the results could contribute to strategies that maximise GHG mitigation potential of SRF as a fossil fuel alternative.

The hypotheses for the main part of this study were that: (i) GHG emissions will be influenced by depth to water table with higher CO₂ and N₂O emissions expected with a lower water table (intermediate aeration) and higher CH₄ emissions at higher water table (saturated) in all

species, (ii) there will be tree species differences in soil GHG fluxes with higher N₂O emissions expected from Sitka spruce and Common alder soils compared to Scots pine (and Grassland) and there will be interactions between tree species and water table depth (iii) microtopography and interactions between microtopography and tree species will modify soil GHG emissions. In order to test whether N limitation was having an impact on N₂O production under particular species a further N addition experiment was carried out using 12 additional mesocosms at the conclusion of the main experiment.

4.3 Materials and methods

4.3.1 Site description and field sampling methods

Soil cores (mesocosms) were collected from Gisburn Forest experimental site in the NW England (54° 1' N; 2°22' W) in May 2014. A full site description and table of soil properties are detailed in chapter 3. As a result of ground preparation prior to forest replanting in 1991 three different microtopographies now exist within the 1.5 m spacing between tree rows; ridges, troughs (furrows) and undisturbed flats. The raised ridges (~50 cm wide) into which trees were planted and adjacent troughs (~25 cm deep × ~50 cm wide) were created by overturning soil on one side the ridge line using a mouldboard plough, and the ~50 cm wide undisturbed flats exist on the other side of the ridge lines. Soil mesocosms were extracted by gently hammering 30 cm deep × 11 cm diameter sections of PVC pipe into the soil, then cutting around the outside with a sharp knife and pulling out with pliers. Two soil mesocosms were extracted from each microtopography in Sitka spruce, Scots pine and common alder habitats (the use of the term 'habitat' throughout this chapter refers to the different land covers including Scots pine, Sitka spruce, common alder and grassland) and from each of the three blocks. Mesocosms were also extracted from the grasslands to use as a reference but these were only taken from undisturbed flats as this is the only existing topography. This resulted in a total of 60 mesocosms. A further three soil mesocosms were collected from the flat microtopography, in each habitat for the N addition experiment. Following extraction, all mesocosms were transported back to the laboratory and stored at 4° C for 2 days prior to the experiment set-up.

4.3.2 Experimental design

Each of the 72 mesocosms comprising the soil in the PVC pipe were weighed, numbered and placed in sturdy 13-L plastic containers (315 mm × 275 mm) (Smithers-Oasis Company, Washington, UK). All mesocosms were kept in a controlled temperature room at 11° C (temperature at which GHG *in situ* emissions peaked, Chapter 3) for the duration of the experiment. To limit the effects of understory plants on soil GHG emissions any visible vegetation (mainly grasses) were carefully removed before starting the experiment. To maintain water tables at one of two set levels (“high” and “low”), 5 mm holes were drilled into the mesocosm at either 3 cm from the surface or 27 cm from the surface. The mesocosms were allowed to equilibrate at 11° C for 1 week prior to water table treatment application to allow time for the soil environment to stabilise and recover following disturbance. Half of the mesocosms from each combination of block and microtopography were assigned to either a high or low water table treatment, and water was added to the buckets to a level that aligned with the top of the drilled holes. Mesocosms were then allowed to equilibrate for 18 days before starting measurements, with water table monitored by daily inspection and maintained by manually topping up with deionised water when required. An additional set of 12 test mesocosms were used to monitor volumetric moisture content (VMC) using a ML2x Theta Probe and HH2 Meter (Delta T Devices, Cambridge, UK) to avoid disturbing the mesocosms from which GHG measurements were made.

4.3.3 Laboratory sampling methods

4.3.3.1 GHG measurements – main experiment

Mesocosms were incubated for a period of 134 days, and GHG fluxes were measured at six time points 18, 26, 40, 54, 69 and 134 days. To measure GHG flux rates, headspace gas samples were taken using the unvented static enclosure method (Livingston and Hutchinson, 1995). Plastic opaque chambers made from Lock & Lock containers cut in half (Lock & Lock, Anaheim, CA, USA, W 110 mm, H 180 mm) were attached to each mesocosm (mean headspace volume $669.65 \pm 12.34 \text{ cm}^3$) and sealed using wrist sections of rubber gloves and layers of duct tape. A 10 mm hole was drilled into each of the Lock & Lock lids and a rubber septum (Sigma Aldrich, St. Louis, MO, USA) was inserted into the hole and the air tightness of the chambers pre-tested. 10-ml headspace gas samples were collected through the rubber

septum using a 20-ml syringe fitted with a 0.5 mm needle, chambers were flushed three times with headspace gas before filling the syringe and transferring to 3-ml pre-evacuated exetainers (Labco, Lampeter, UK). Sampling was carried out over a 45 minute enclosure period with samples taken immediately after sealing the lid, then at three fifteen minute intervals. Gas samples were analysed for CO₂, CH₄ and N₂O concentrations on a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) with flame ionization detector and electron capture detector equipped with a poropack Q column operated at 60° C with an argon carrier gas. Certified gas standards (Air Products, Crewe, UK) within the range of the samples being analysed (497, 1063, 4110 ppm CO₂, 1.07, 3.03, 10.26 ppm CH₄ and 0.41, 0.99 and 2.04 ppm N₂O) were used to calibrate the GC. Gas fluxes (CO₂, CH₄ and N₂O) were calculated using the approach of Holland et al. (1999) by plotting the linear accumulation of each gas over the 45 minute enclosure period.

4.3.3.2 Destructive sampling

At the end of the main water table manipulation experiment the 60 mesocosms used for this experiment were destructively sampled and the depth of the soil organic layer of each was measured. Subsamples were used to calculate bulk density (BD), gravimetric moisture content (GMC), water filled pore space (WFPS) and for available N analysis (NH₄⁺-N and NO₃⁻-N). Gravimetric moisture was determined from a quarter mesocosm subsample placed in an oven at 105° C for 24 hours. BD was calculated using these values of moisture loss following methods in the GB Countryside Survey (Emmett et al., 2008; Reynolds et al., 2013). Inorganic N concentration was determined by extraction with 6% KCl extraction. The extracts were analysed for NH₄⁺-N and NO₃⁻-N colourimetrically using an AQ2 discrete analyser (Seal Analytical, Southampton, UK).

4.3.3.3 Nitrogen addition experiment

A subset of twelve mesocosms were retained at the end of the main experiment and a further N addition experiment conducted to test relative N limitation across habitats. These mesocosms were from low water table treatments and from the flat microtopography. Ammonium nitrate in water solution (58-ml) was added at a concentration equivalent to monthly (winter months) N deposition at Gisburn Forest (4.16 kg N ha⁻¹) (APIS, 2014). Headspace GHG samples were collected over a 45 minute enclosure period, using the same

method as above, on 18 occasions: 1 hour before N addition, 1 hour after, 4 hours after, then after 1, 2, 3, 4, 5, 6, 7, 11 and 14 days. The gas samples were stored in 3-ml pre-evacuated exetainers and analysed by GC (as above). At the end of this additional experiment these twelve mesocosms were also destructively sampled, and analysed as per the sixty original mesocosms for BD, GMC, soil organic layer depth, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$.

4.3.4 Data processing and statistical analyses

The effects of time, SRF tree species, water table, microtopography, and their interactions, on GHG fluxes were examined in a fully factorial design using linear mixed-effect models. Core was included as a random effect in these models to account for the repeated measures made on each mesocosm. Grassland mesocosms were not included in these analyses since they were represented by only one microtopography. However, a separate analyses including both SRF and grassland mesocosms but using only the 'flat' microtopography tested whether there was a difference between these land uses. Measures made on mesocosm soils at the conclusion of the main experiment were used to test the effects of species, water table and their interaction on GMC, WFPS, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, and the effects of species, microtopography and their interaction on BD and the depth of the soil organic layer.

The effects of N addition, and whether soils under different species responded differently to N addition, were assessed by testing a 'before-after' dummy variable for N addition and its interaction with species, respectively. The 'before-after' variable omitted the sampling times 1 h, 4 h and 24 h after N addition, when GHG fluxes were considered to represent the disturbance of water and N addition.

4.4 Results

4.4.1 Water table depth and microtopography effects on GHG fluxes across SRF species

4.4.1.1 CO₂ efflux

Time had a significant effect on CO₂ efflux during incubation (Table 4.1). CO₂ efflux rate decreased over the 134 day incubation time across all tree species and the grassland, microtopographies and water table treatments, from a mean of 30.33 mg CO₂-C m⁻² h⁻¹ (Appendix A.3.), to 21.16 mg CO₂-C m⁻² h⁻¹ (Appendix A.3.). Tree species had a significant effect on soil CO₂ efflux (Table 4.1). Sitka spruce mesocosms showed higher soil efflux rates (34.21 ± 1.55 mg CO₂-C m⁻² h⁻¹) compared to Scots pine (24.09 ± 0.90 mg CO₂-C m⁻² h⁻¹) and common alder (22.45 ± 1.06 mg CO₂-C m⁻² h⁻¹) mesocosms. Only Sitka spruce mesocosms had a higher CO₂ efflux rate than the grassland (27.21 ± 1.73 mg CO₂-C m⁻² h⁻¹).

Table 4.1. Summary statistics for the effects of tree species, sampling time, water table depth, microtopography (ridge, trough, flat) and their interactions on soil GHG fluxes. Bold indicates values are significant at $P < 0.05$.

	CO ₂	CH ₄	N ₂ O
time	P = 0.004	P = 0.859	P = 0.613
tree species	P = 0.025	P = 0.230	P < 0.001
water table	P = 0.905	P = 0.026	P = 0.036
topography	P = 0.810	P = 0.402	P = 0.335
time : species	P = 0.123	P = 0.707	P = 0.170
time : water table	P = 0.527	P = 0.286	P < 0.001
time : topography	P = 0.103	P = 0.189	P = 0.475
species : water table	P = 0.665	P = 0.361	P = 0.043
species : topography	P = 0.239	P = 0.815	P = 0.294
water table : topography	P = 0.158	P = 0.810	P = 0.841
time : species : water table	P = 0.403	P = 0.612	P = 0.149
time : species : topography	P = 0.215	P = 0.898	P = 0.062
time : water table : topography	P = 0.345	P = 0.328	P = 0.581
species : water table : topography	P = 0.068	P = 0.520	P = 0.949
time : species : water table : topography	P = 0.551	P = 0.547	P = 0.545

There was no overall or interactive effects of water table depth on CO₂ emissions from SRF mesocosm soils (Table 4.1), despite there being higher CO₂ efflux at low water table depth in the Sitka spruce, similar emissions in Scots pine at both water table depths, and higher emissions at high water table in common alder mesocosms (Fig. 4.1A).

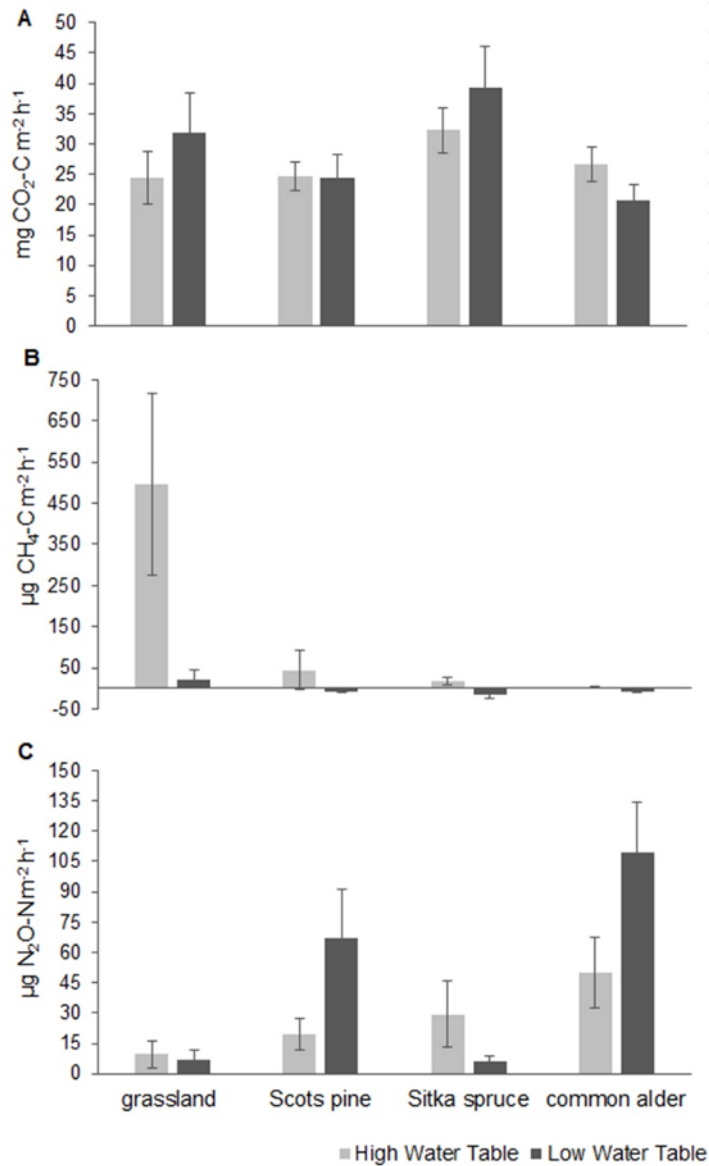


Figure 4.1. Soil GHG fluxes for all habitats. (A) CO₂, (B) CH₄, (C) N₂O, at high (3 cm below surface) and low water table (27 cm below surface) depths. Error bars represent standard error. n= 3 for Grassland mesocosms; n = 9 for tree species mesocosms.

Microtopography also had no effect on CO₂ efflux and no significant interactions with other factors (Table 4.1). There were, however, apparent trends in the interaction between water table depth and microtopography (Fig. 4.2A). At high water table CO₂ efflux was higher from ridges (31.17 ± 2.36 mg CO₂-C m⁻² h⁻¹) compared to flats and troughs which had similar mean efflux rate (24.75 ± 0.87 and 24.15 ± 1.56 mg CO₂-C m⁻² h⁻¹, respectively). Whereas, at low water table CO₂ efflux rates were higher from troughs (31.18 ± 2.36 mg CO₂-C m⁻² h⁻¹), a trend that appears to be largely driven by Sitka spruce, and effluxes from flats and ridges were the same (26.01 ± 1.14 and 26.47 ± 2.05 mg CO₂-C m⁻² h⁻¹, respectively) (Fig. 4.2A).

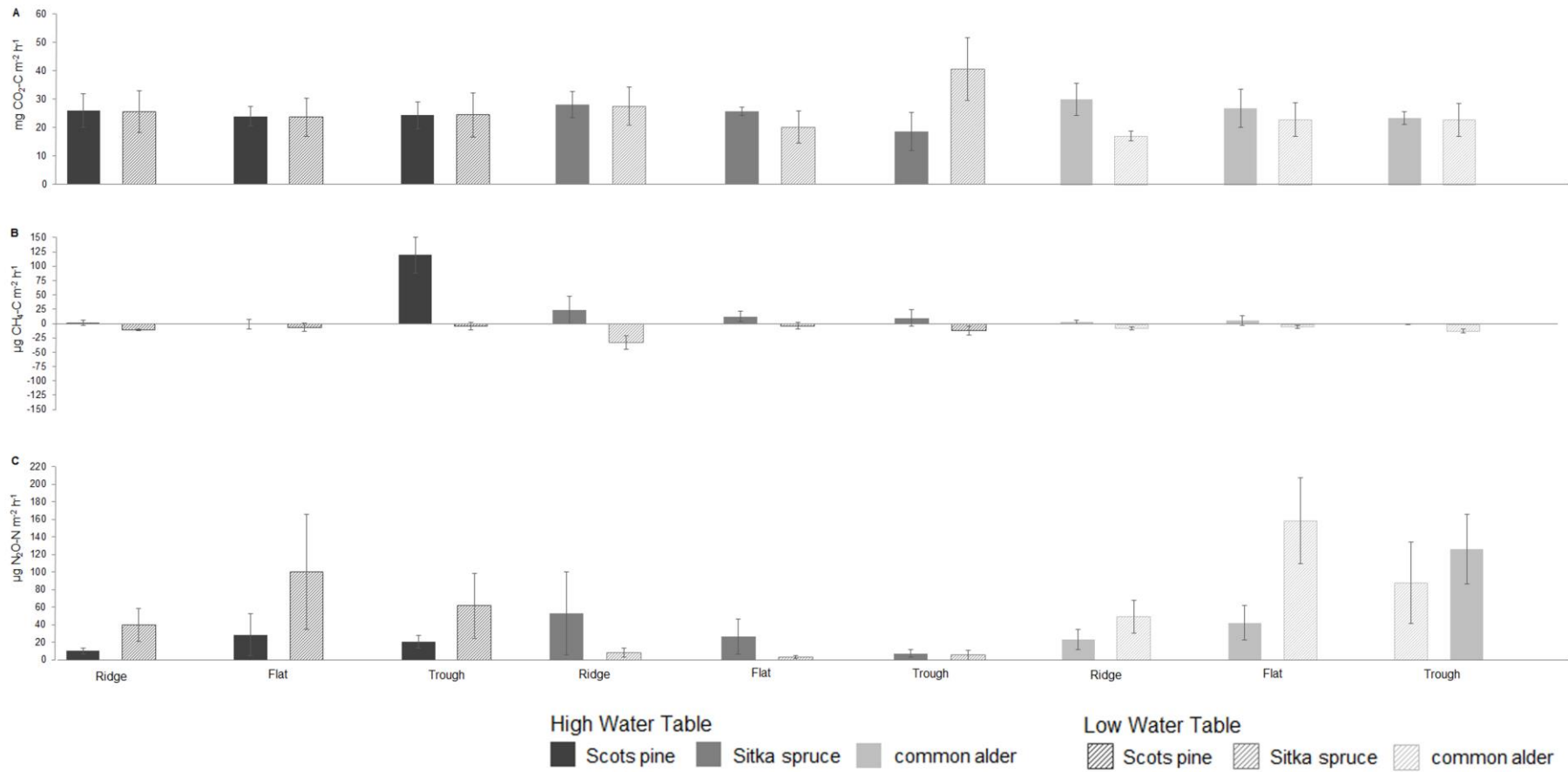


Figure 4.2. Soil GHG fluxes for all habitats. (A) CO₂, (B) CH₄, (C) N₂O, at high and low water table from each microtopography (ridges, flats and troughs). Error bars represent standard error. n= 3.

4.4.1.2 CH₄ fluxes

There was no effect of time on soil CH₄ fluxes (Table 4.1) and over the 134 days of incubation there was mean net CH₄ uptake in the Sitka spruce ($-1.80 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) and common alder soils ($-0.69 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) and Scots pine mesocosms ($-4.76 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$). Grassland soils ($177.06 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) were not significantly different from the flats of the tree species mesocosms (Appendix A.4.).

Water table had an effect on CH₄ flux (Table 4.1), and in SRF overall there was net CH₄ production from soils with high water table ($21.76 \pm 19.47 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) and net uptake in soils at low water table depth ($-10.52 \pm 3.73 \mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) (Fig. 4.1B). There was no interaction between water table depth and tree species (Table 4.1). In comparison, net CH₄ production was measured in all grassland mesocosms irrespective of water table treatment, but CH₄ production rates were much greater at high water table (Fig. 4.1B). At low water table there was still net CH₄ production from the grassland mesocosms, compared to uptake in the tree species mesocosms with rates declining in the order of Sitka spruce > common alder > Scots pine (Fig. 4.1B).

Overall, microtopography had no significant effect on soil CH₄ fluxes (Table 4.1). There were no interactions between species, water table and microtopography, however interesting trends existed (Fig. 4.2B). In the common alder mesocosms at high water table CH₄ production was greatest from the flat microtopography and lowest from troughs, with ridges intermediate (Fig. 4.2B). Whereas, at low water table, where net CH₄ uptake was measured, uptake declined in the order of troughs > ridges > flats (Fig. 4.2B). CH₄ production was highest from troughs in Scots pine mesocosms at high water table compared to ridges, and even at high water table there was net CH₄ uptake in the undisturbed flats. At low water table depth in Scots pine there was net CH₄ uptake, with rates decreasing in the order of ridges > flats > troughs (Fig. 4.2B). The pattern was again different in Sitka spruce mesocosms where highest rates of CH₄ production at high water table were measured in ridges lowest in troughs

and flats intermediate (Fig. 4.2B). Greatest uptakes rates at low water table were found in ridges followed by troughs, with lowest uptake in flats (Fig. 4.2B). The effect of microtopography in grassland could not be tested as only flats existed.

4.4.1.3 N₂O fluxes

As with CH₄ flux, there was no effect of time on overall N₂O fluxes in mesocosms (Table 4.1) (Appendix A.5.). There was, however, an interactive effect of time and water table on N₂O flux rates (Table 4.1). N₂O flux generally decreased over the duration of the study at high water table (T1: 27.19 ± 6.97, T2: 50.95 ± 13.65, T3: 32.51 ± 6.28, T4: 28.98 ± 9.69, T5: 22.53 ± 6.37 and T6: 21.42 ± 7.74 µg N₂O-N m⁻² h⁻¹), but increased over the duration of the study at low water table treatment ((T1) 23.23 ± 6.00, (T2) 34.89 ± 8.82, (T3) 47.68 ± 7.16, (T4) 50.37 ± 10.61, (T5) 56.81 ± 11.95 and (T6) 82.76 ± 15.94 µg N₂O-N m⁻² h⁻¹) (Appendix A.5.).

Tree species affected N₂O fluxes significantly (Table 4.1) and net N₂O emissions were measured in all species, decreasing in the order of common alder (73.72 ± 5.93 µg N₂O-N m⁻² h⁻¹) > Scots pine (46.77 ± 5.37 µg N₂O-N m⁻² h⁻¹) > Sitka spruce (14.48 ± 3.92 µg N₂O-N m⁻² h⁻¹). All tree species mesocosms had higher mean N₂O flux rates than the grassland over the course of the experiment (10.73 ± 2.78 µg N₂O-N m⁻² h⁻¹). Water table depth had an effect on N₂O fluxes (Table 4.1) and net emissions were measured from both water table treatments, but with higher flux rates from mesocosms with low water table treatment (60.98 ± 16.98 µg N₂O-N m⁻² h⁻¹) compared to high water table treatment (33.07 ± 13.85 µg N₂O-N m⁻² h⁻¹) (Fig. 4.1C). This water table effect on N₂O fluxes was also influenced by species, with common alder and Scots pine having greater fluxes at low water table compared to high water table, whereas Sitka spruce fluxes were greater at high water table than at low water table depth (Fig. 4.1C). N₂O emissions in grassland flat mesocosms were significantly lower than common alder ($P < 0.01$) but not Sitka spruce or Scots pine (Appendix A.5.).

There was no overall effect of microtopography on N₂O fluxes (Table 4.1). There were also no interactions between microtopography, water table or tree species (Table 4.1).

There were, however, trends between microtopography and tree species. For instance, N₂O fluxes in common alder mesocosms at low water table were highest from flats and lowest from ridges, whereas at high water table N₂O emissions were highest from troughs and lowest from ridges. (Fig. 4.2C). In Sitka spruce mesocosms at both high and low water table treatments, N₂O emissions declined in the order of ridges > flats > troughs. Whereas, in Scots pine mesocosms, at both high and low water table, the highest emissions were from flats and lowest from ridges (Fig. 4.2C).

4.4.1.4 Available N concentrations

At the end of the main experiment there were differences in available N levels between high and low water table treatments (Fig. 4.3). Overall (considering the mean of all habitats) both NH₄⁺ and NO₃⁻ were higher in the low water table mesocosms compared to the high water table mesocosms (Fig. 4.3), however the effect of water table was only significant for NO₃⁻ ($F_{1,50} = 28.84$, $p < 0.001$). Although there were no significant effects on NH₄⁺ concentrations or interactions with habitat ($F_{3,50} = 1.30$, $p = 0.28$) or water table depth ($F_{1,50} = 0.51$, $p = 0.48$) there were differences observed between species at low water table. Highest NH₄⁺ was found in the Sitka spruce soils and the Scots pine and common alder soils had similar concentrations (Fig. 4.3A). All tree species mesocosms had higher available NH₄⁺ than the grassland (Fig. 4.3A). In contrast, there was a significant effect of habitat ($F_{3,50} = 3.89$, $p = 0.01$) on concentrations of NO₃⁻ which were lowest in Sitka spruce mesocosms at low water table and increased in the order of Sitka spruce < grassland < Scots pine < common alder (Fig. 4.3B). At high water table there was almost no NO₃⁻, likely to be as result of complete denitrification, and only small amounts measured in Scots pine, common alder and Sitka spruce, and none in the grassland mesocosms (Fig. 4.3B). NH₄⁺ concentrations were similar across all tree species and only slightly lower in the grassland (Fig. 4.3A).

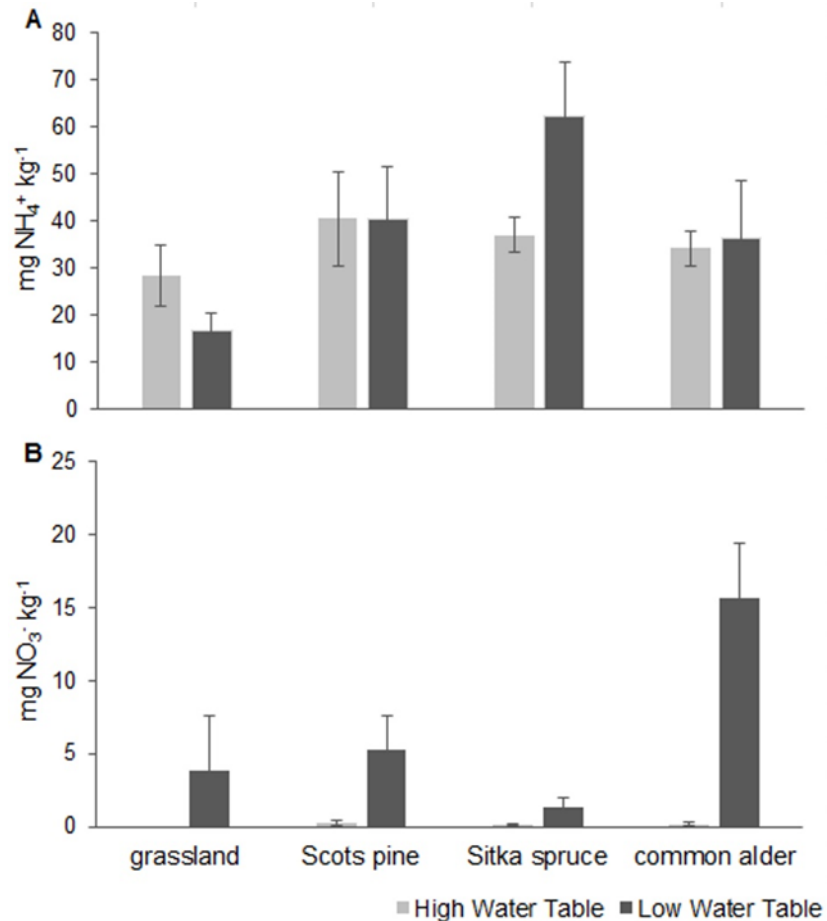


Figure 4.3. Soil available N at high and low water table depths in mesocosms at the end of the main experiment (141 days). (A) ammonium NH₄⁺, (B) nitrate NO₃⁻. Error bars represent standard error. n= 3 for Grassland mesocosms; n = 9 for tree species mesocosms.

4.4.1.5 Factors affected by water table depth

Gravimetric moisture content (GMC) was affected by water table depth ($F_{1,49} = 6.05$, $p = 0.02$) with higher GMC in mesocosms at high water table compared to low water table. There was no difference in GMC between species ($F_{3,49} = 2.51$, $p = 0.07$) or any interaction between species and water table depth ($F_{3,49} = 1.23$, $p = 0.31$). Water filled pore space (WFPS) was also influenced by water table depth ($F_{1,49} = 8.47$, $p = 0.01$) with 100.20 ± 14.07 (%) WFPS measured in high water table mesocosms, and 66.79 ± 6.81 (%) in low water table mesocosms. There was no effect of species ($F_{3,49} = 0.49$, $p = 0.67$), or any interaction between species and water table depth ($F_{3,49} = 1.65$, $p = 0.19$) on WFPS.

4.4.1.6 Factors affected by microtopography

There were differences in soil bulk density (BD) with microtopography ($F_{2,43} = 3.14$, $p = 0.05$) declining in the order of flats ($0.45 \pm 0.03 \text{ g cm}^{-3}$) > troughs ($0.36 \pm 0.03 \text{ g cm}^{-3}$) > ridges ($0.34 \pm 0.03 \text{ g cm}^{-3}$). Tree species had no effect on soil BD ($F_{2,43} = 2.12$, $p = 0.13$) and there was no interactive effect of species and microtopography ($F_{4,43} = 0.38$, $p = 0.82$). There was no difference in depth of soil organic layer between microtopographies ($F_{2,42} = 0.62$, $p = 0.54$), however there was a highly significant effect of species ($F_{2,42} = 19.86$, $p < 0.001$) on depth of the organic layer and there was also an interaction between species and topography ($F_{4,42} = 2.83$, $p = 0.04$). Overall, soil organic layer was deepest in Sitka spruce mesocosms ($3.92 \pm 0.32 \text{ cm}$), followed by grassland ($3.17 \pm 0.70 \text{ cm}$) and Scots pine ($3.03 \pm 0.33 \text{ cm}$), but more shallow in common alder mesocosms (only $1.61 \pm 0.25 \text{ cm}$). In the Scots pine and common alder mesocosms the soil organic layer was deepest on ridges ($3.33 \pm 0.21 \text{ cm}$, $2.17 \pm 0.64 \text{ cm}$) whereas in the Sitka spruce mesocosms it was deepest in troughs ($4.83 \pm 0.75 \text{ cm}$).

4.4.2 N addition experiment

4.4.2.1 CO₂ fluxes

There was no difference in soil CO₂ efflux between habitats ($F_{3,8} = 1.81$, $p = 0.22$). Soil CO₂ efflux was higher in the grassland ($31.00 \pm 0.39 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$) and Sitka spruce ($30.81 \pm 0.29 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$) mesocosms than those in Scots pine ($14.82 \pm 0.05 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$) and common alder ($12.37 \pm 0.14 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$) mesocosms (Fig. 4.4A). There was a small decrease in CO₂ efflux following the addition of N (1 hour after addition) in the grassland and Scots pine mesocosms with the rates beginning to increase from the decreased level after 4 hours. There was also no overall effect of N addition on CO₂ efflux ($F_{1,116} = 2.47$, $p = 0.12$) or any interaction between CO₂ efflux and tree species in SRF ($F_{3,116} = 0.99$, $p = 0.41$) owing to the small increase in Sitka spruce mesocosms but absence of change in Scots pine, and a small decrease in common alder (Fig. 4.4A).

4.4.2.2 CH₄ fluxes

Similar to CO₂ response there were no differences in CH₄ flux between habitats ($F_{3,8} = 3.03$, $p = 0.09$) and there was no effect of N addition ($F_{1,116} = 1.51$, $p = 0.22$), or any interaction between tree species and N addition ($F_{3,116} = 0.58$, $p = 0.63$). The addition of N to the Scots pine mesocosms did not significantly increase net CH₄ uptake, but the mean net efflux was $-2.44 \pm 0.99 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ before compared to $-6.32 \pm 0.81 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ after N addition. The Sitka spruce mesocosms remained a net sink for CH₄, though the sink strength decreased from $-15.43 \pm 1.57 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ to $-7.22 \pm 1.45 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ (Fig. 4.4B). Despite a peak in CH₄ uptake 2 days after N addition, the common alder mesocosms showed similar CH₄ uptake rates before and after N addition ($-4.57 \pm 0.71 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ and $-4.52 \pm 1.80 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$, respectively) (Fig. 4.4B). In grassland mesocosms there was an initial reduction in CH₄ emissions following N addition followed by, two peaks followed after 2 days and 4 days. After this point CH₄ emissions returned to a steady rate that was approximately double that prior to N addition ($12.96 \pm 0.14 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ to $26.98 \pm 1.54 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$) (Fig. 4.4B).

4.4.2.3 N₂O fluxes

There was a significant effect of N addition on N₂O emissions ($F_{1,116} = 13.96$, $p = 0.003$) with an overall 142% increase in N₂O emissions following N addition from 39.94 ± 7.74 to $96.58 \pm 26.34 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. As for CO₂ and CH₄, there was no effect of habitat on soil N₂O fluxes ($F_{3,8} = 2.16$, $p = 0.17$), nor any interaction between habitat and N addition ($F_{3,116} = 0.26$, $p = 0.86$). Despite the lack of significant interaction between habitat and N addition, there was interesting variation in magnitude of response between habitats. In all habitats there was a reduction in N₂O emissions following N addition for ~ 24 hours, after which time rates started to increase again. Following N addition the highest emissions of N₂O were measured in the Scots pine mesocosms where rates more than doubled from $113.01 \pm 9.00 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ to $247.29 \pm 7.22 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$, followed by grassland where emissions more than

trebled from 31.59 ± 4.52 to $106.16 \pm 3.68 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$. However, the greatest proportional response was in the Sitka spruce mesocosms where N_2O emissions increased from 7.53 ± 1.58 to $66.37 \pm 2.71 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$, despite N_2O emission rates being lower than those from Scots pine and grasslands. N_2O emissions also increased in common alder mesocosms from 35.69 ± 1.19 to $73.47 \pm 4.39 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ (Fig. 4.4C).

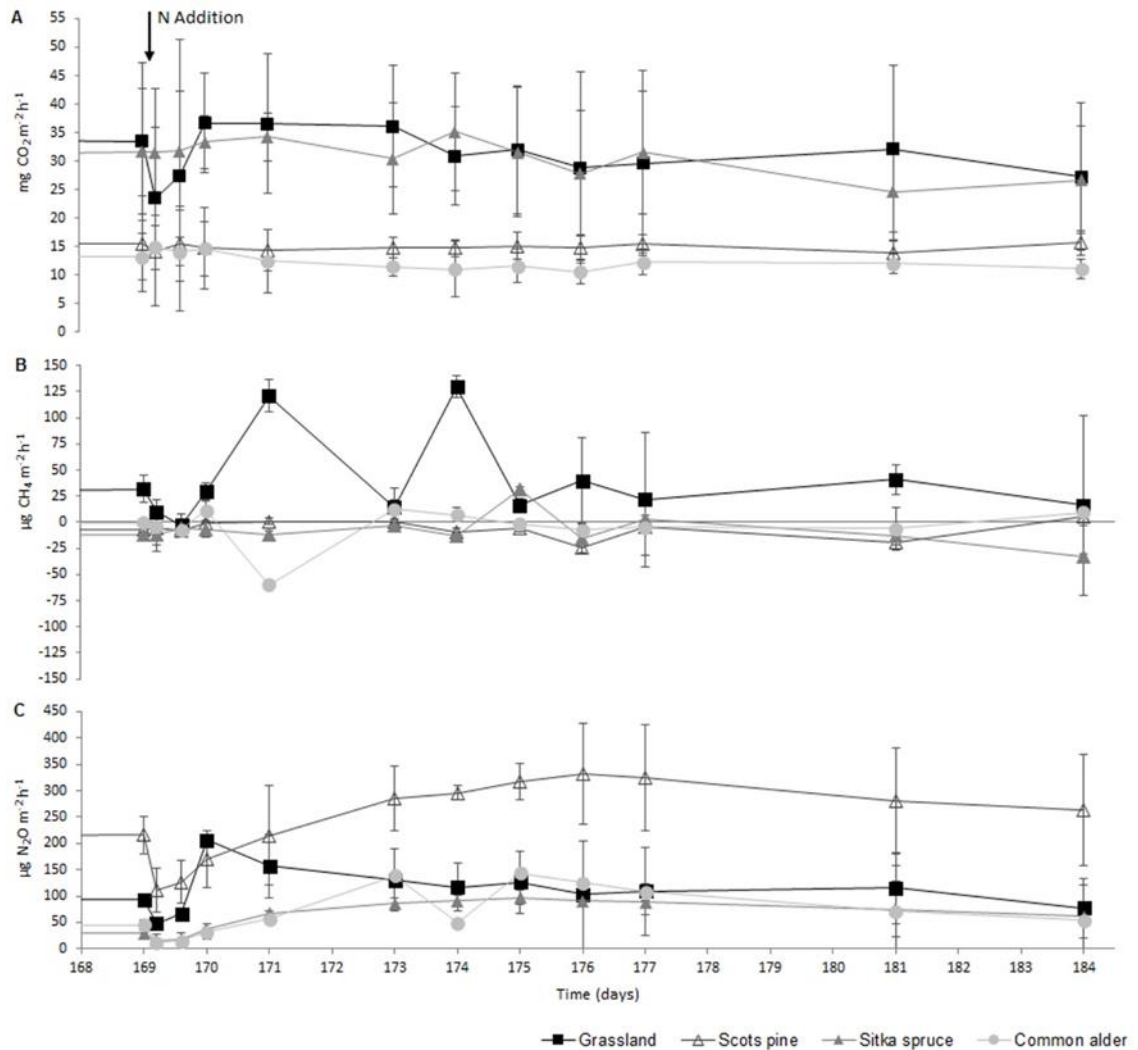


Figure 4.4 Soil GHG fluxes for all habitats. (A) CO_2 , (B) CH_4 , (C) N_2O from an additional set of 12 mesocosms (undisturbed flat microtopography only) following the addition of $\text{H}_2\text{O} + \text{NH}_4\text{NO}_3$ equivalent to $4.62 \text{ kg N ha}^{-1}$ on day 169 (arrow indicates timing of N addition). Three GHG measurements were made on day 169, 1 hour before N addition, 1 hour after N addition, and a further measurement 4 hours after N addition. Error bars represent standard error. $n=3$.

4.4.2.4 Available N concentrations

Seven days after the end of the N addition experiment (184 days from start of main experiment) NH_4^+ was highest in the Sitka spruce mesocosms. Similar concentrations of NH_4^+ were measured in common alder and Scots pine and the lowest in grassland. There was large variation in grassland, common alder and Scots pine results (Fig. 4.5). Concentrations of NO_3^- at the end of the experiment were considerably higher in the common alder mesocosms compared to the grassland, Sitka spruce and Scots pine (Fig. 4.5). There was less variability in the NO_3^- measurements compared to the NH_4^+ measurements from the same samples (Fig. 4.5). Comparing NH_4^+ concentrations after N addition to those measured at the end of the main experiment for mesocosms of flat microtopography at low water table, there was a decrease from beginning to end in all species (Fig. 4.3, Fig. 4.5).

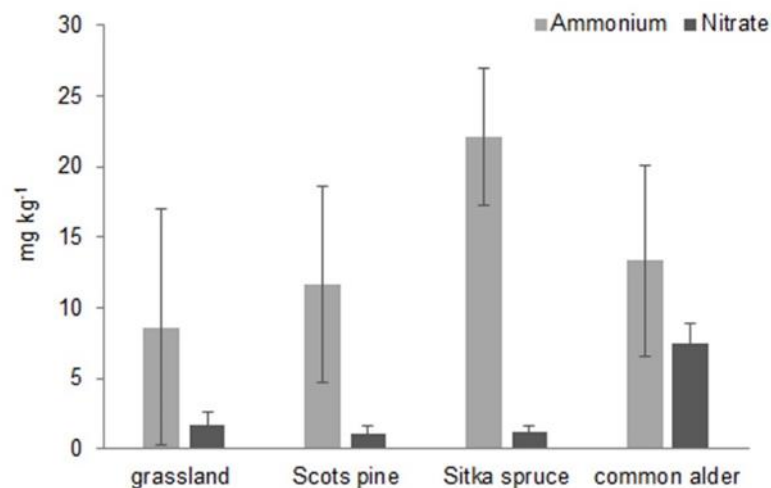


Figure 4.5. Soil available N (Ammonium NH_4^+ and Nitrate NO_3^-) in mesocosms at the end of the N addition experiment (184 days). Error bars represent standard error. $n = 3$.

4.5 Discussion

GHG fluxes are highly variable in field studies of LUC as a result of differences in physical, environmental and climatic conditions, both spatially and temporally (Gundersen et al., 2012; Kern et al., 2012; Zona et al., 2013). It is also difficult to quantify soil heterotrophic respiration due to the contribution of autotrophic respiration to overall CO₂ efflux, which in forests in particular is difficult to partition. One means of overcoming these field related issues is to extract soil core mesocosms from the field and maintain them intact under controlled conditions in the laboratory (Kirschbaum, 2006; Schaufler et al., 2010). Soils are initially disturbed, but less so than when using sieved homogenous soils and having experimental control allows one to independently manipulate variables to better understand what is driving soil GHG emissions (Jungkunst et al., 2008).

4.5.1 Effects of tree species and water table on soil GHG fluxes

For this study two water table treatments were used to investigate the effects of saturated soils (3 cm below surface) and soils of intermediate water table depth (27 cm below surface) on soil-atmosphere GHG exchange under different tree species. Saturated conditions were chosen due to the known relationship between soil CH₄ emissions and high soil moisture (Smith et al., 2003; Dinsmore et al., 2009). Intermediate water table was chosen as N₂O emissions have been known to peak when water table depths are in the region of 15–35 cm below the surface (Jungkunst et al., 2004), or with soil moisture within the range of 40–50% volume (Ball et al., 2007).

In support of the first hypothesis, water table depth had a significant effect on N₂O and CH₄ fluxes in mesocosms. N₂O emissions from the tree species mesocosms were within the range estimated for temperate forests (0.01-8.07 kg N₂O-N ha⁻¹ y⁻¹; Dalal & Allen, 2008) for both low water table (5.35 ± 1.49 kg N₂O-N ha⁻¹ y⁻¹) and high water table (2.91 ± 1.21 kg N₂O-N ha⁻¹ y⁻¹) treatments. Although considerably higher, Jungkunst et al. (2008) also recorded higher N₂O emissions at lower water table from

laboratory incubated mesocosms collected from a temperate forest in Germany (-40 cm depth = 23.14 ± 1.30 , -5cm depth = 7.27 ± 1.39 kg N₂O-N ha⁻¹ y⁻¹). The higher N₂O emissions measured at intermediate water table depth are also in agreement with the conceptual model of Davidson et al. (2000) which predicts highest N₂O production as a result of incomplete denitrification at intermediate WFPS. This is expected because nitrifier activity is greatest at a moderate WFPS (up to 60%), while denitrifier activity increases greatly with soil WFPS > 60% (Bateman & Baggs, 2005) as result of decreased O₂ diffusion into the soil (Ruser et al., 2006). When water table depth is 'intermediate' (i.e. 15–35 cm), aeration in the surface layers increases leading to an elevated rate of decomposition releasing more N by mineralisation which acts as a substrate for N₂O production (Freeman et al., 1996). Although there was no correlation between WFPS and soil N₂O fluxes in this study it is worth noting that at high water table WFPS was on average 100%, whilst at intermediate water table the WFPS was $66.79 \pm 6.81\%$. Therefore, in this study it is possible that at both water table depths N₂O production was mostly a product of incomplete denitrification, but rates were higher at intermediate water table as conditions were more favourable for production. There were some mesocosms with low water table where WFPS was lower than 60% and, therefore, nitrification may have been the primary pathway for N₂O production in these cores. These likely pathways are further supported by the concentrations of soil available N found in this study. Across all habitats there were higher concentrations of NH₄⁺ than NO₃⁻, suggesting that nitrification in these soils may be limited with NH₄⁺ not being used efficiently, or it could also suggest that nitrification is the dominant pathway for N₂O production. Johnson (1992) suggests that high concentrations of NH₄⁺ in soils almost always lead to high rates of nitrification. Low concentrations of NO₃⁻ are not unusual as it is known to be less strongly absorbed in the soil system and is more susceptible to leaching in most soils (Johnson & Turner, 2014). The higher concentration of NO₃⁻ measured in the common alder soils is likely to be as a result of N₂ fixation by the actinomyorrhizal nodules (*Frankia* sp.) of alder root tips and subsequent conversion of NH₃/NH₄⁺ to NO₃⁻ in the surrounding soil (Reay et al., 2005).

The second hypothesis was also partly supported for N₂O with different effects of water table on N₂O found in soils under different species. At low water table (intermediate aeration) N₂O emissions were higher in common alder and Scots pine mesocosms compared to high water table mesocosms. Higher N₂O emissions at 'intermediate' water table depth were expected as increased aeration facilitates more efficient release of substrate necessary for incomplete denitrification (Ball et al., 2007). In Sitka spruce mesocosms, it was therefore surprising that N₂O emissions were greater, although very variable, at high water table (saturated) compared to low water table. However, N₂O emissions were very small from Sitka spruce mesocosms at both water table depths (high: 29.36 ± 16.18; low: 6.10 ± 2.35 µg N₂O-N m⁻² h⁻¹), compared to field measurements (Chapter 3) of 83.92 ± 10.81 µg N₂O-N m⁻² h⁻¹. This suggests that N₂O emissions from Sitka spruce soils are driven by factors that were not controlled in the mesocosm experiment, such as N input to the soil via atmospheric deposition. It was hypothesised that there would be higher N₂O emissions from Sitka spruce mesocosms and common alder mesocosms, which were expected as a response to higher concentrations of available N likely to be found under these species. This was found to be true for common alder but not for Sitka spruce. As discussed above, higher concentrations of NO₃⁻ were measured in soils under common alder as a likely result of N fixation and have been recorded in field studies previously (Reay et al., 2005; Lu et al., 2015). As N deposition is known to be higher in dense canopied coniferous plantations (Erisman & Draaijers, 2003; de Vries et al., 2007; Michopoulos et al., 2007) it was expected that there would be more available N in the Sitka spruce soils, this was true for NH₄⁺ but not NO₃⁻. A recent study by Carnol and Bazgir (2013) measured a significantly higher annual return of NO₃⁻-N via throughfall under Norway spruce compared to six other species, including common alder, at a site in Belgium with a similar atmospheric N deposition rate, climate, and soil type to Gisburn forest. This research, and the high N₂O emission rates measured in the field (Chapter 3), might suggest that NO₃⁻ in the Sitka spruce soil as a result of throughfall, is efficiently reduced to N₂O via incomplete denitrification at intermediate moisture. Alternatively, NO₃⁻ may be reduced to NO (not measured in this study) in saturated

soils as a result of complete nitrification, which could explain the low concentrations found in the mesocosm soils and the lack of N₂O flux overall.

In comparison to the grassland reference mesocosms (low water table, 0.84 ± 0.58 kg N₂O-N ha⁻¹ y⁻¹ and high water table, 0.57 ± 0.46 kg N₂O-N ha⁻¹ y⁻¹) it appears that LUC to SRF has had an adverse effect on net N₂O emissions irrespective of depth to water table. This confirms the findings of the earlier work in the field (Chapter 3) and indicates that afforestation of grassland in this soil type, regardless of species related differences leads to an increase in soil N₂O emissions.

Water table depth was the only significant determinant of CH₄ fluxes, with net emissions measured at high water table and net consumption at low water table, across all tree species. This was as expected as CH₄ is produced by methanogens under anaerobic, low oxygen (O₂) conditions, and CH₄ is consumed by methanotrophs when soils are well aerated. The importance of water table depth on CH₄ fluxes has been reported in other field studies (von Arnold et al., 2004; Ball et al., 2007; Jungkunst et al., 2008; Zona et al., 2013; Zenone et al., 2015) and laboratory studies (Dinsmore et al., 2009). The CH₄ emissions rates measured in this study are low compared to some others published for forest soils at high water table (e.g. 50-100 µg CH₄-C m⁻² h⁻¹ at water table 5 cm below surface, Jungkunst et al., 2008) but not as low as the 3.42 µg CH₄-C m⁻² h⁻¹ from a wet Swedish beech and Norway spruce site measured by Gundersen et al. (2012). Net CH₄ uptake rates were also low compared to other published rates for European forests (e.g. mean ± SE: -46 ± 20 µg CH₄-C m⁻² h⁻¹, Skiba et al., 2009) and compared to the estimate for global temperate forests in general (mean 41.07 µg CH₄-C m⁻² h⁻¹, Dalal & Allen, 2008). They are, however, similar to rates previously published for this site (mean ± SE: -5.05 ± 7.61 µg CH₄-C m⁻² h⁻¹, McNamara et al., 2008). The relatively low CH₄ uptake may be due to the suppression of this process by high levels of N deposition (44.38 kg N ha⁻¹ yr⁻¹ for deposition years 2010-2012) (APIS, 2015) at this site, and as a result of N fixation in the common alder soils (Butterbach-Bahl et al., 1998; Reay & Neadwell, 2004; McNamara et al., 2008). The grassland reference mesocosms were net producers of CH₄ at both water table

depths indicating that LUC from grassland to SRF could have a positive sink effect for atmospheric CH₄ when water table is not at the surface. As for N₂O, this finding on CH₄ confirms earlier work (Chapter 3), and it is also in agreement with the findings of McNamara et al. (2008) who also measured net CH₄ emissions from the grasslands at Gisburn Forest (mean ± SE: 39.0 ± 36.68 μg CH₄-C m⁻² h⁻¹) compared to net uptake under common alder, Scots pine and Sitka spruce. Since the grassland soil always tend to be wetter in field conditions it may be that the microbial community are adapted to these conditions.

Support for hypotheses one was not found with regard to soil CO₂ efflux which was similar under both water table treatments. However, in agreement with the first part of hypothesis two, there were significant differences in CO₂ flux between species, with higher fluxes measured from Sitka spruce mesocosms, compared to Scots pine or common alder overall. This pattern was similar to the findings from the field measurements of Chapter 3, but respiration rates from the mesocosms were considerably lower compared to field measurements (e.g. Sitka spruce: mesocosms, 34.21 ± 1.55 mg CO₂-C m⁻² h⁻¹, field 97.66 ± 4.41 mg CO₂-C m⁻² h⁻¹). This is likely to be as a result of understorey vegetation removal and subsequent dark respiration, and reduced root inputs and nutrient supply in the mesocosms which may have led to a reduction in soil microbial activity (Fang & Moncrieff, 2001; Schaufler et al., 2010).

4.5.2 Does microtopography modify soil GHG fluxes?

Although we found no significant effects of microtopography or interactions with tree species, there were trends which indicated that microtopography could modify the influence of water table and tree species on GHG fluxes. Similar to the findings of Saiz et al. (2006), this study measured highest CO₂ efflux from Sitka spruce troughs, but only at low water table depth. Saiz et al. (2006) attributed their findings from across multiple stands to the deeper organic layer in the furrows (troughs) (e.g. 3.9 ± 0.3 cm in 31 year old Sitka spruce stand) compared to ridges and flats, which contains a high proportion of total fine root biomass. In this study the soil organic layer in the Sitka spruce troughs (4.8 ± 0.2 cm) was also deeper than in ridges or flats. Spatial

variability of soil CO₂ efflux has been previously related to the soil organic layer thickness. In a Canadian boreal forest study an increase in soil CO₂ efflux was positively related to the thickness of the soil organic layer as a result of microtopographical differences (Rayment & Jarvis, 2000). It is possible, therefore, that the CO₂ efflux measured from Sitka spruce trough mesocosms may have been driven by the decomposition of severed fine roots (as a result of soil coring) in the deep soil organic layer, and increased aeration at 'intermediate' water table depth. However, Ball et al. (2007) measured higher mean CO₂ fluxes from ridges in a Sitka spruce chronosequence in Northumberland, and attributed this to water table depths being lower in ridges compared to ditch sides (flats) or ditches (furrows/troughs). This finding of Ball et al. (2007) is in agreement with this study because at high water table depth higher CO₂ efflux was also measured from Sitka spruce ridges, further suggesting an interaction between microtopographical-related differences in soil properties and water table.

Microtopographical effects, and interactions with tree species were also examined for soil N₂O fluxes. Consistent patterns were limited as results were very variable between species at different water table depths. Only Scots pine soils had a consistent pattern at both water table depths, with higher N₂O emissions from flat microtopography. This consistency suggests that N₂O production pathways in Scots pine soils are partly affected by microtopography, but any effect is further modified by water table depth as a result of its effect on aeration. In Sitka spruce mesocosms, despite the lower fluxes compared to other tree species, there was a trend towards higher N₂O emissions from ridges at high water table. This may be related to the higher CO₂ flux also measured from Sitka spruce ridges at high water table explained above, and to the increased C input from severed decomposing fine roots. An increase in soil C inputs could have an indirect effect on soil N₂O emissions as heterotrophic microorganisms responsible for soil N transformation depend on a supply of available organic C (Hodge et al., 2000). Other than assuming that there is a greater abundance of fine roots in ridges (i.e. closer to the tree) it is difficult to explain why

there would be increased C as a result of fine root decomposition in any one particular microtopography. Considering the common alder mesocosms, from which N₂O fluxes were greatest overall, the pattern at low water table was the same as for Scots pine declining in the order of flats > troughs > ridges. This could be attributed to the abundance and distribution of N-fixing actinomyorrhizal nodules in flats and troughs compared to ridges. In a study by Rytter (1989) nodules were found up to 35 cm away from the tree stump, but were most abundant closer to the stump, this study was however conducted on young alder stands (4 years old), and distribution may be different in older stands. These species and water table trends highlight the importance of measuring soil-atmosphere GHG exchange across different microtopographies in order to capture this aspect of spatial variability.

4.4.3 N addition

The low N₂O emissions measured from Sitka spruce mesocosms, as explained previously, was the reason behind undertaking the supplementary N addition experiment. Using a subset of 12 mesocosms (3 from each habitat) from flat microtopography and low water table only, NH₄⁺ NO₃⁻ was added at a rate equivalent to one month's N deposition for Gisburn Forest. The expectation was that N addition would stimulate the microbial processes responsible for N₂O production as a result of increased substrate availability in Sitka spruce soils, and further increase N₂O production in Scots pine and common alder soils. This was based on previous published studies and an extensive meta-analysis which found that N addition in this form significantly increased soil N₂O emissions (Zhang et al., 2008; Liu & Greaver, 2009; Wang et al., 2014). Nitrogen addition had a significant effect on soil N₂O emissions overall, with a mean increase of 142% relative to pre-addition levels, but there was no significant difference in N₂O emissions between species. This overall N₂O response to N addition is similar to that of Wang et al. (2014) who found a 125% increase in soil N₂O emissions following N addition to forest plots in China in the wet season (WFPS > 60%). In their wet season, the peak rate of N₂O emissions was 97.4 µg N₂O-N m⁻² h⁻¹ following N addition, which is similar to the mean of this study 96.58

$\pm 26.34 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ after N addition. Mesocosm N_2O flux rates after N addition were higher than the *in situ* rates of Chapter 3 ($35.92 \pm 3.68 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$), further confirming the effect of N addition on soil N_2O emissions.

Although there were no significant differences in habitat response to the N addition, or any interaction between habitat and N addition, there was a varied magnitude of response between species that is worth mention. N_2O emissions were highest from the Scots pine mesocosms, both before and after the addition of N, indicating that soil conditions for N_2O production were already favourable and the added N substrate further stimulated production (by 118%). Even though the highest emissions were from Scots pine mesocosms, it was the Sitka spruce mesocosms that showed the greatest relative response with a 780% increase in N_2O emissions after N addition. Despite this high relative response, Sitka spruce soil N_2O emissions were still lower than those measured *in situ* (Chapter 3), $66.37 \pm 2.71 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ versus $83.92 \pm 10.81 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$. An explanation for this shortcoming may be due to a lack of ectomycorrhizal fungi in the mesocosms which were possibly severed during the soil coring process. Certain N-tolerant fungi, such as *Paxillus involutus* and *Tylospora fibrillosa*, which can dominate the microbial biomass in acidic temperate forest soils (Smith & Read, 2008), have been found to produce N_2O through nitrate reduction under low oxygen conditions (Prendergast-Miller et al., 2011). These species of fungi are known to be highly competitive when inorganic N concentrations are high (Carfrae et al., 2006). As atmospheric N deposition is expected to be higher in the Sitka spruce habitat, it is possible that a proportion of N_2O emitted from these soils comes from ectomycorrhizal fungi.

At the end of the N addition experiment concentrations of inorganic N were measured from the mesocosms. Across all habits there were higher concentrations of NH_4^+ than NO_3^- detected, with the highest concentration found in the Sitka spruce mesocosms. As ammonium is oxidised to nitrate in the three step process of nitrification (Thomson et al., 2012) it is unlikely that this was a primary pathway for N_2O production in the N addition mesocosms. With the exception of the common

alder mesocosms, NO_3^- concentrations were very low in the mesocosms at the end of the experiment. This might suggest that incomplete denitrification was the primary pathway for N_2O production as this process involves the enzymatic reduction of NO_3^- . Comparing available soil inorganic N at the end of the N addition experiment to concentrations measured *in situ* from cores collected in the same sampling month (May 2014), values were similar for all habitats except common alder. For example, Sitka spruce soils contained $21.51 \pm 1.23 \text{ mg NH}_4^+ \text{ kg}^{-1}$ and $0.15 \pm 0.01 \text{ mg NO}_3^- \text{ kg}^{-1}$ *in situ*, compared to $22.12 \pm 4.83 \text{ mg NH}_4^+ \text{ kg}^{-1}$ and $1.21 \pm 0.43 \text{ mg NO}_3^- \text{ kg}^{-1}$ in the mesocosms. This similarity in inorganic N concentrations for Sitka spruce might further suggest that NO_3^- is used very efficiently in this habitat and that N_2O emissions are a product of high N deposition and incomplete denitrification.

In this study N addition had no effect on soil CO_2 efflux. Although it was expected that the effect of increased N substrate availability would increase microbial activity and subsequent CO_2 emissions in the short-term as a result of increased C decomposition (Brumme & Beese, 1992; Contosta et al., 2011), this finding is not unusual. Many studies have found that N addition has not had an effect on soil CO_2 efflux (Allison et al., 2008; Ambus & Robertson, 2006; Castro et al., 1994b; Micks et al., 2004; Mo et al., 2007). Even long-term *in situ* N addition experiments in forests have had no effect on soil CO_2 emissions (Koehler et al., 2009; Koehler et al., 2012; Krause et al., 2013). Furthermore, some studies have found that N addition reduces CO_2 efflux (Bowden et al., 2004; Burton et al., 2004; Janssens et al., 2010; Micks et al., 2004). There was also no significant effect of N addition on CH_4 fluxes. It was expected that the addition of N might decrease CH_4 uptake rates due to the known suppressive effect that increased N can have on CH_4 oxidation (Castro et al., 1995; Sitaula et al., 1995; MacDonald et al., 1996). This lack of response might be as result of the low dose of N applied to the mesocosms, and the short duration of the N addition experiment.

4.6 Conclusions

The results of this study demonstrate that water table depth can modify the pattern of soil-atmosphere CH₄ and N₂O exchange but has little impact on CO₂ efflux. Therefore, consideration should be given to the impact that temporal fluctuations in water table will have on the magnitude and direction of soil GHG fluxes. Tree species had a significant effect on soil GHG fluxes overall, with highest CO₂ efflux from Sitka spruce, and highest N₂O emissions from common alder soils. These species differences highlight the need for careful consideration in species selection for SRF, to ensure that tree species will offer the greatest C and GHG savings for a given soil type. Furthermore, these findings suggest that in forest sites N₂O may need to be measured across representative microtopographies, in order for more accurate calculation of GHG balances.

Chapter 5. Discussion and conclusions

A substantial amount of grassland across Europe could be converted to SRF for biomass production in the near future (Zenone et al., 2015). This LUC will be driven by the requirement to achieve renewable energy and GHG emissions targets and to find a sustainable alternative to fossil fuel combustion. As a result of this, it is important to understand the implications of planting trees into grassland, especially for soil-atmosphere GHG exchange and soil C storage, to ensure that maximum mitigation potential is reached. Although conventional forestry has been practiced and studied for many years, little is known about the effects of growing high density plantations of trees on shorter rotations. As tree species have differential effects on the soil environment it is important to carefully select species for SRF that can offer the greatest benefits. This study investigated the effects of LUC from grassland to different SRF species on the soil-atmosphere exchange of the three primary GHGs (CO₂, N₂O and CH₄) and the important mechanisms underlying these effects.

Chapter 2 - Soil GHG potentials were measured under controlled laboratory conditions using sieved soil sub-samples incubated in Wheaton bottles (*sensu* Reay et al., 2001; Reay et al., 2005). These soils were collected from under a variety of coniferous and broadleaved tree species along with soils from adjacent paired grasslands from six different UK sites. Associated soil physico-chemical properties were measured and microbial community composition was assessed by phospholipid fatty acids (PLFA) profiling.

Chapter 3 - The soil-atmosphere exchange of CH₄, N₂O and net CO₂ (which includes dark respiration of the ground vegetation) was monitored *in situ* under rough ungrazed grassland and monocultures of Scots pine, Sitka spruce and common alder, over 16 months at the Gisburn Forest Experimental site. The relative importance of a range of soil physical and chemical variables for GHG fluxes were assessed.

Chapter 4 – The effects of Scots pine, Sitka spruce and common alder SRF on soil GHG fluxes were further investigated in a medium-term (184 days) manipulation

study and an additional N addition experiment using intact mesocosms collected from the Gisburn Forest Experimental site. This experiment focused on how water table depth (3 cm vs 27 cm), microtopography (flats, ridges and troughs), and the interactions between them could modify tree species effects on soil GHG fluxes.

5.1 Land use change to SRF

5.1.1 Soil CH₄ fluxes

Key findings

1. Under controlled laboratory conditions there was a small net CH₄ uptake in both grassland and SRF soils but no significant differences between these land uses (Chapter 2).
2. There were net CH₄ emissions from grassland soils and net uptake in all tree species soils *in-situ*, but the difference between grassland and SRF was not significant (Chapter 3).

Soil CH₄ fluxes were very small throughout this study but there was a consistent pattern of net CH₄ uptake in SRF soils compared to net emissions in the grassland soils. This finding was anticipated as temperate forest soils are known to act as a significant sink for atmospheric CH₄ (Bowden et al., 2000). Even though this trend was clear, there was no significant difference in soil CH₄ fluxes between grassland and SRF in the GHG potential study of Chapter 2. This may be partly a result of the experiment being optimised for CO₂ production. There was a significant difference in soil CH₄ fluxes between grasslands and SRF *in situ* (Chapter 3) but soil uptake rates across all species were low (mean 0.9 kg CH₄-C ha⁻¹ y⁻¹) compared to the mean for temperate forests (mean 3.6 kg CH₄-C ha⁻¹ y⁻¹, Dalal & Allen, 2008). These low soil CH₄ uptake rates were higher than those measured by McNamara et al. (2008) at Gisburn Forest over a 12 month period in 1999–2000 (mean uptake of 0.4 kg CH₄-C ha⁻¹ y⁻¹). This suggests that the soil CH₄ sink potential increases with plantation age, which is consistent with a range of other studies (Smith et al. 2000). There were differences in

CH₄ uptake rates between tree species types (Chapter 2) with higher uptake in broadleaved soils compared to coniferous. There were also differences between tree species (Chapter 3) with higher uptake in Scots pine and lowest in Sitka spruce soils, but again these differences were not significant. This trend is contrary to the norm, where CH₄ uptake rates are generally higher in broadleaved soils compared to coniferous (Jang et al., 2006; Skiba et al., 2009). The reason for higher uptake in the Scots pine soils might be due to CH₄ uptake in common alder and Sitka spruce soils being suppressed as a result of greater N availability (Reay et al., 2005). Overall, the evidence from this thesis suggests that LUC from grassland to SRF would have a positive effect on the soil sink strength for CH₄.

5.1.2 Soil N₂O fluxes

Key findings

1. There was a trend towards higher N₂O emissions from SRF and, within SRF, greater N₂O emissions from soils under coniferous species compared to soil under broadleaved species under controlled laboratory conditions. Differences in soil N₂O flux between grassland and SRF soils were, however, not significant (Chapter 2).
2. In the field N₂O emissions were significantly higher from SRF soils compared to grassland soils and, within SRF, Sitka spruce soil emitted significantly more N₂O compared to Scots pine and common alder soil (Chapter 3).
3. In the laboratory nitrogen addition had a significant positive effect on N₂O emissions with the highest relative response from Sitka spruce mesocosms, but highest absolute rates of N₂O emissions were from Scots pine soils (Chapter 4).

Until recent years N₂O emissions from forest soils were considered negligible compared to those from fertilised agricultural soils which can emit up to ~18 kg N₂O-N ha⁻¹ y⁻¹ (Dobbie et al., 1999). Now it is understood that forest soils could be

significant sources of N₂O with emission rates up to 8.07 kg N₂O-N ha⁻¹ y⁻¹ (Dalal & Allen, 2008). In Chapter 2, N₂O fluxes were very small as a result of the experiment being optimised for CO₂ production but there was a trend towards higher net N₂O emissions from coniferous soils compared to broadleaved, and unchanged compared to grasslands. In contrast, N₂O emissions from the in-situ study (Chapter 3) were significantly higher from SRF soils (4.1 kg N₂O-N ha⁻¹ y⁻¹) compared to the negligible emissions of the grasslands (0.2 kg N₂O-N ha⁻¹ y⁻¹). Considering that most temperate forests emit less than 0.5 kg N₂O-N ha⁻¹ y⁻¹ (Brumme et al., 1999), the rate of emission at Gisburn Forest is comparably high and likely a result of the soil environment. Further, N₂O emissions were higher from Sitka spruce soils and, with a mean rate of 7.4 kg N₂O-N ha⁻¹ y⁻¹, at the upper end of the range estimated by Dalal & Allen (2008). Other studies have measured higher rates of N₂O emission from soils under coniferous species compared to deciduous (Barrena et al., 2013). Zechmeister-Boltenstern et al. (2002) measured N₂O emissions of 4 kg N₂O-N ha⁻¹ y⁻¹ from a mature beech forest in Austria and attributed this high emission rate to high atmospheric N inputs (~ 35 kg N ha⁻¹ y⁻¹). This is also likely to be the reason for high N₂O emissions at Gisburn Forest (Chapter 3) where total atmospheric N deposition is ~44 kg N ha⁻¹ y⁻¹. Based on the outcome of the single site study of Chapter 3, and other published studies, LUC from grassland to SRF can lead to increased soil N₂O emissions which vary depending on tree species.

5.1.3 Soil C and CO₂ efflux

Key findings

1. Under controlled laboratory conditions CO₂ efflux was significantly reduced in soils from SRF compared to soils from grasslands (Chapter 2).
2. *In-situ* chambers in field revealed net CO₂ efflux (including dark respiration) was significantly higher from grassland soils compared to SRF soils and, within SRF, higher from Sitka spruce soils compared to Scots pine and common alder soils (Chapter 3).

3. In mesocosms where vegetation had been removed, CO₂ efflux was highest from Sitka spruce soils compared to Scots pine and common alder soils, but with no effect of water table depth on CO₂ efflux (Chapter 4).

It has been identified that land uses that are high in SOC stocks, such as grasslands could be particularly susceptible to LUC to bioenergy crops compared to low C soils such as croplands (Poeplau et al., 2011). However, to date, there has been limited research into the transition from grasslands to bioenergy as the focus has been on arable land transitions (Harris et al., 2015). This LUC effect will be greatly influenced by the type of energy crop that is to be planted and in the case of planting SRF, a positive effect would be expected. This is mainly because planting of trees is known to generally promote soil C storage (Vesterdal et al., 2012) and because grasslands are known to be smaller sinks for CO₂ compared to forests (Raich & Tufekcioglu, 2000). While this may be an oversimplification due to the effects of other factors on soil C and soil-atmosphere GHG exchange such as plantation age, species type, land management, understorey abundance and composition and soil conditions, the recent meta-analysis carried out by Harris et al. (2015) concluded that of all bioenergy transitions, the largest uncertainty is in quantifying the impacts of LUC from grasslands to SRF on soil GHG emissions.

In this thesis we provide support for the expectation that LUC to SRF could result in reduced soil CO₂ emissions and increased soil C (Chapter 2). Examining soils from across six different sites we found that the magnitude of change in soil CO₂ efflux was influenced by tree species type (coniferous or broadleaved). However, this magnitude of change was also modified depending on whether CO₂ flux was calculated on a soil mass or a soil C mass basis, especially with regard to coniferous soils. A greater reduction in the broadleaved soils was shown when expressing soil CO₂ efflux on a soil mass basis, whereas a greater reduction in coniferous soils was shown when soil CO₂ efflux was expressed in relation to the amount of C in the soil. This highlights the importance of careful consideration when deciding how to express CO₂ efflux before

drawing conclusions about LUC effects. The greater reduction in CO₂ efflux on a mass of C basis from the coniferous soils (Chapter 2), strengthens the findings of Keith et al. (2015), who measured higher soil C stock in coniferous soils compared to broadleaved soils across multiple sites (including these six sites).

The more focused single site approach of Chapter 3 also suggested a reduction in soil CO₂ emissions following transition from grassland to SRF, though this effect may be overestimated because the measured CO₂ flux may also result from the dark respiration of ground vegetation. However, this overall reduction in soil CO₂ efflux from SRF is in agreement with the findings of Raich & Tufekcioglu (2000) who analysed data from 10 different paired-site studies on the effects of LUC from grassland to forest and found on average a 20% reduction in soil CO₂ efflux from forests. Chapter 3 also identified species-specific differences, with a smaller reduction of CO₂ emissions in Sitka spruce soils compared to common alder and Scots pine. Other authors have found tree species differences in soil CO₂ efflux, however, efflux rates have generally been reported as being higher from broadleaved soils compared to coniferous as a result of more labile litter inputs and faster nutrient turnover (Borken & Beese, 2005; Berger et al., 2010; Vesterdal et al., 2012). The rate of CO₂ efflux from the Sitka spruce soils in situ ($97.66 \pm 4.41 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$, Chapter 3) were similar to those measured by Saiz et al. (2006) from Sitka spruce soils in Ireland ($103.69 \pm 10.22 \text{ mg CO}_2\text{-C m}^{-2} \text{ h}^{-1}$), and therefore appear to be robust. Higher CO₂ efflux from Sitka spruce soils is likely to be as a result of the higher C concentration and C stock in the soil (Chapter 3, Keith et al. 2015).

Considering the data derived from the in situ study of Chapter 3, LUC from rough grassland to SRF is likely to result in reduced annual soil net CO₂ emissions or no change depending on tree species. As other similar studies on transitions to SRF do not exist it is difficult to make a direct comparison between the outcome of this study and others. However, LUC from grasslands to SRC for bioenergy has been reported to increase in soil CO₂ efflux by $6.7 \text{ t ha}^{-1} \text{ y}^{-1}$, whereas transitions from grasslands to perennial grasses for bioenergy have resulted in a decrease in efflux of $0.8 \text{ t ha}^{-1} \text{ y}^{-1}$

(Harris et al., 2015). Based on the sites measured in this study, it would appear that LUC from grassland to SRF for bioenergy has a greater impact on soil CO₂ efflux than SRC and a similar impact to perennial grasses. Soil CO₂ efflux in forests has been found to be dependent on plantation age, with a trend towards efflux increasing with stand age (Ball et al., 2007). In this thesis, plantations studies ranged from 16 to 23 years (23 year old site was in its 2nd rotation), and therefore, the magnitude of change in soil CO₂ emissions could be related to plantation age.

5.2 Sources of variation in field GHG fluxes

5.2.1 Water table depth

Key findings

1. Water table depth and interactions between tree species and water table were important drivers of GHG fluxes in the field (Chapter 3).
2. There were net soil CH₄ emissions at high water table and net uptake at low water table, with no effect of tree species on CH₄ fluxes (Chapter 4).
3. Both common alder and Scots pine had higher soil N₂O emissions at low water table compared to at high water table. In contrast, Sitka spruce soils emitted more N₂O at high water table compared to at low water table (Chapter 4).

Water table depth can have a significant effect on soil-atmosphere GHG exchange due to its influence on soil aeration and subsequently water filled pore space, and on the supply of substrate and oxygen to the soil microbial community (Davidson et al., 1998; Ball et al., 2007; Dinsmore et al., 2009). Planting trees can lead to water table draw down, well below the depth of the prevailing vegetation (Smith et al., 2003), due to their high demand for water (McKay, 2011). Water table drawdown has been shown to lead to increased decomposition rates and subsequent CO₂ efflux (Chivers et al., 2009), increased N₂O emissions (Martikainen et al., 1993; Huttunen et al., 2003), and increased CH₄ uptake (Hughes et al., 1999). This high requirement for water is likely to be greater in SRF systems than in conventional forest systems due to the

shorter rotation lengths and faster growth rates (McKay, 2011). Previous studies have shown that tree water use is directly related to growth rate and declines with age (Vertessy et al., 1995; Watson et al., 1999; Almeida et al., 2007).

In Chapter 3 of this thesis, water table depth was found to be an important regulator of soil CH₄ fluxes in the field, where net emissions were measured under the permanently saturated grasslands, compared to net uptake under SRF. This relationship was further investigated in the laboratory water table manipulation experiment of Chapter 4, where water table depth had the greatest effect of soil CH₄ fluxes of all measured variables. The outcome showed that at high water table CH₄ was emitted from all soils but at a far greater magnitude from grasslands, and at low water table CH₄ uptake occurred in all tree species soils but not in the grassland. This relationship between water table and CH₄ fluxes in forest systems has been found by others (von Arnold et al., 2005; Ball et al., 2007; Jungkunst et al., 2008; Zenone et al., 2015) and is expected due to the known requirement of CH₄ oxidising methanotrophic bacteria for aerobic conditions (Hanson & Hanson, 1996). This evidence suggests that the high water demand of SRF compared to grassland, and its subsequent influence on water table depth could lead to an increase in the soil sink potential for atmospheric CH₄.

Water table and its interaction with species explained a considerable amount of the variation (11%) in N₂O fluxes *in situ* (Chapter 3). For example, in the Sitka spruce soils, where the highest net N₂O emission rates were measured overall, emissions peaked when water table was at 20–30 cm below the surface, but were negligible when the water table was >40 cm below the surface. This trend suggests that incomplete denitrification is the primary pathway for N₂O production in these soils as emissions are very sensitive to moisture concentrations and peak at intermediate moisture conditions (> 60%), whereas nitrification favours drier soil conditions (< 60%) (Bateman & Baggs, 2005). In the grassland soils *in situ* (Chapter 3) where soils were permanently saturated, N₂O emissions were negligible across the entire 16 month sampling period. In the mesocosm water table manipulation experiment (Chapter 4)

this relationship between intermediate water table depth (27 cm below surface) and N₂O emissions was further tested. In contrast to the outcome of the field study, at intermediate water table depth in this study N₂O emissions peaked in common alder and Scots pine soils, whereas N₂O emissions were very small for the Sitka spruce soils. Through a further additional N addition experiment it was determined that this observation was as a result of N limitation in the Sitka spruce soils which were usually subjected to high atmospheric N inputs in the field. Once again, N₂O emissions were much lower from grassland soils compared to all SRF species in the mesocosm study (Chapter 4). This influence of 'intermediate' water table depth on N₂O emissions in forests has been measured and modelled by others (Davidson et al., 2000; Ball et al., 2007). For example, Jungkunst et al. (2004) measured lower N₂O emissions at a water table depth of 65–75 cm but higher emissions when the water table was intermediate at 15–35 cm. The outcome of the experiments carried out in this thesis together with other published data on forests, suggests that LUC from grasslands to SRF could result in increased N₂O emissions which could be further modified by the direct influence of trees on water table depth.

Soil CO₂ efflux was not greatly influenced by water table depth (Chapters 3 and 4) despite the known relationship between respiration and soil moisture (Davidson et al., 1998). In Chapter 4, CO₂ efflux rates were similar at both high and low water table depth across all species, including the grassland. This is likely to be as a result of CO₂ efflux being more a product of C input quality and differences in decomposition rates between species (Chapter 3). In Chapter 2, LUC from grassland to SRF lead to decreased CO₂ efflux as a result of changes in the soil microbial community composition and reduced soil pH. Therefore, although soil moisture is an important regulator of respiration, LUC from grassland to SRF and its impact on water table depth is likely to be less important for soil CO₂ efflux.

5.2.2 Spatial effect of microtopography

Key findings

1. A substantial proportion of variation in soil GHG fluxes in the field was explained by spatial factors, accounting for up to ~20% in the case of CH₄ (Chapter 3).
2. While microtopography and its interaction with tree species and water table depth was not significant in laboratory mesocosms, there were trends of specific patterns of N₂O fluxes for each tree species, which were influenced by water table depth (Chapter 4).

The issue of spatial variability as a result of topographical differences in soil environments was a shortcoming identified by Butterbach-Bahl et al. (2013) with regard to field based N₂O measurements in their influential paper on processes and controls of N₂O emissions in soils. In Chapter 3, a large proportion of the variability in soil GHG fluxes *in situ* was attributed to spatial effects, which encompassed the random effects of block and plot. However, a proportion of this spatial variability could have been partly due to microtopography, and as GHG sampling chambers were installed at random in the field it was difficult to test for the effect of microtopography independent of block and plot. Thus, the effect of microtopography on soil GHG fluxes was tested systematically in the Chapter 4 mesocosm experiment. While no significant effects of microtopography or any interactions with water table and tree species were found, there were trends that indicated that microtopography could modify the influence of water table and tree species on soil GHG fluxes. For example, soil CO₂ efflux was higher from Sitka spruce troughs compared to from ridges or furrows, at low water table. This finding is shared with Saiz et al. (2006) who attributed higher CO₂ efflux from Sitka spruce troughs to the deeper organic layer which contains a greater abundance of fine roots. A study by Rayment and Jarvis (2000) confirmed that soil CO₂ efflux is positively related to organic layer thickness. The soil organic layer in the Sitka spruce troughs was also thicker than under other

microtopographies (Chapter 3) and, therefore, this is also a likely explanation for the higher CO₂ efflux measured. Few consistent patterns were found in mesocosms for N₂O fluxes from different microtopographies (Chapter 4) with fluxes varying greatly with water table depth for each species. Both Scots pine and common alder soils emitted higher rates of N₂O from mesocosms collected from flat microtopography at low water table (i.e. when emission rates were highest). In the case of the common alder, this outcome may be related to the distribution of N-fixing actinomycorrhizal nodules (Rytter, 1989). Whereas, the Sitka spruce soils emitted highest N₂O at high water table from ridges. Ball et al. (2007) measured higher CO₂ efflux from Sitka spruce ridges compared to flats or troughs in Harwood Forest, Northumberland, and attributed this to variation in depth to water table between microtopographies. This may also be the case for N₂O, and although water table depth was controlled across all mesocosms (Chapter 4), the soil environment in the mesocosms may have been already conditioned in the field leading to increased N₂O production. Microtopography had the least effect on soil CH₄ fluxes despite spatial effects explaining ~ 20% variation in CH₄ fluxes in the field (Chapter 3). These highlighted trends, together with the findings of others, suggest that LUC from grassland (flat topography only) to SRF, using the pre-planting method that creates ridges, troughs and flats, could have further implications for the magnitude of change in CO₂ efflux and N₂O emissions.

5.3 Limitations and future study

This thesis and the work of Keith et al. (2015) has demonstrated that SRF in the UK has the potential to deliver GHG and C savings at the field scale. For example, converting rough grasslands to SRF can lead to soil C savings in the region of 1400 kg C ha⁻¹ y⁻¹ depending on tree species and the soil environment (Keith et al., 2015). The work in Chapters 2 and 3 may be considered to provide support for the expectation that LUC to SRF can result in reduced soil CO₂ emissions. However, the *in situ* field measurements of soil CO₂ efflux also includes dark respiration, which can account for up to 50% of overall CO₂ flux (Epron et al., 2001; Subke et al, 2006). Therefore, it is possible that CO₂ effluxes have been over-estimated making it difficult to determine the LUC effect of converting rough grassland to SRF. The comparison of tree species and the grassland may be influenced by the respiring biomass of ground vegetation and further work in these experimental plots should remove ground vegetation throughout the measurement campaign. In addition, the removal or exclusion of roots from the areas where soil respiration is measured would allow for partitioning of soil respiration into heterotrophic and autotrophic respiration. This would provide a more robust understanding of the effect of LUC to SRF on soil CO₂ fluxes.

Despite our consistent N₂O flux results, it was not possible to determine the ultimate biological responses accounting for differing N₂O production in the field or in the laboratory studies. Soil N₂O emissions increased under SRF compared to the original grassland land use (Chapters 3 and 4), with differences between species. This increase in N₂O emissions was more pronounced in coniferous soils compared to broadleaved, and in particular from Sitka spruce soils. Ectomycorrhizal fungi are often abundant in the organic litter layers of acidic coniferous forest soils. Under laboratory conditions, Prendergast-Miller et al. (2011) showed that ectomycorrhizal fungi extracted from Sitka spruce root tips could produce N₂O. To date, this has not been demonstrated under field conditions, and therefore with regard to soils under Sitka spruce at Gisburn Forest it would be interesting to investigate if N₂O is being produced by ectomycorrhizal fungi in the deep litter layer. This might be done by

using techniques such as litter layer removal or by inserting ectomycorrhizal exclusion cores and taking GHG samples in the absence/presence of the litter layer and ectomycorrhizal fungi.

Tree species can directly and indirectly affect water table depth due to their species specific variation in; demand for water, depth and composition of the soil litter layer, canopy densities and subsequent interception of rainfall, and as a result of varied root architecture. This influence on water table depth can affect soil microbial activity, which is known to be sensitive to soil moisture which in-turn influences soil-atmosphere GHG exchange. N₂O production is particularly sensitive to water table depth with N₂O produced as a result of nitrification at lower water table and at intermediate water table as a result of incomplete denitrification. Future work should investigate whether the soil N₂O fluxes found from soils *in-situ* and in laboratory mesocosms from Gisburn Forest are a product of nitrification or incomplete denitrification under different tree species.

Beyond carrying out further experimental work, it is also recognised that the results of the work in this thesis need to be put into the wider context of the overall GHG balance including downstream processes for producing energy (Whitaker et al., 2010; Rowe et al., 2013). In this work studies were carried out on existing plantations and it is likely that additional GHG savings might be achieved through improved methods of cultivation. For example, choosing the most beneficial tree species for a given soil and climate and/or through improved planting methods which deliver reduced disruption of soils and their carbon stocks.

The experiments throughout this thesis focussed on a defined range of tree stand ages (16-23 years) and additional work could further reduce uncertainty in GHG assessments by following transitions from establishment through to the harvesting stage, and through multiple rotations. For example, it has been reported that large amounts of soil N₂O can be emitted during the establishment phase of tree species such as Poplar (Zona et al. 2013; Zenone et al., 2015). Beyond C and soil-atmosphere GHG exchange, the sustainability of SRF needs to be confirmed through a wider

assessment of impacts on other ecosystem services such as water availability and quality. Currently there is a major shortfall in available data to carry out such a study (Milner et al., 2015). However, further field-based studies capturing soil GHG fluxes from establishment to harvesting and into multiple rotations would further strengthen the outcome of this work.

Chapter 6. Reference List

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Chapter 7. Appendix

A.1. Phospholipid Fatty Acid (PLFA) Extraction and Analysis

PLFAs were extracted as part of the total lipid extract of freeze-dried soils (ca. 2 g dry weight) using a modified Bligh-Dyer extraction (White et al., 1979). In brief, soils were placed in glass culture tubes and extracted with dichloromethane (DCM)/MeOH/citrate buffer (5:10:4 v/v/v; citrate buffer 0.15M adjusted to pH 4 using NaOH pellets). Soil/solvent solutions were placed in an ultrasonic bath (20 min), and then centrifuged (5 min, 1900 rpm). The supernatant was transferred to a second glass tube, and the soil extracted with fresh solvent (x 2). The organic and aqueous phases of the combined solvent extracts were broken with the addition of 2 ml citrate buffer and 2-ml DCM, the organic layer removed, and the aqueous layer washed with 3 x 2 ml DCM. Combined DCM extracts were blown down under N₂ (heating block, 40° C).

PLFAs were separated from other lipids using an aminopropyl solid phase extraction cartridge (Phenomenex). The column was conditioned with DCM/IP (2:1, 6-ml), and the total lipid extract added to the column dissolved in a small amount of the same solvent. Neutral lipids were eluted with 8-ml 2:1 DCM/Iso-propyl alcohol (IPA), followed by elution of the acidic lipids with 6-ml 2% glacial acetic acid in diethyl ether. Polar lipids, including PLFAs, were eluted using 8-ml MeOH. The polar lipid fraction was blown down under N₂ (heating block, 40° C).

Prior to saponification of the polar lipids, nonadecane in known concentration was added to all samples, to enable quantification of PLFAs. Samples were saponified with the addition of 2-ml 0.5M NaOH in MeOH, heated at 70° C for 90 min. Samples were acidified to pH 2 using 0.5M HCl, and lipids extracted with 3 x 2 ml DCM. Samples were reduced to dryness using N₂.

Fatty acids were methylated using boron trifluoride-methanol complex (14% w/v, 30 µl, 70° C, 10 min) and the reaction quenched with water. The resultant fatty acid methyl esters (FAMES) were extracted into hexane (3 x 0.5-ml) and the solvent

concentration adjusted as appropriate for analysis by gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

GC analysis was carried out on an Agilent 6890 GC fitted with a CP-Sil 5CB fused silica capillary column (60 m x 0.32 mm ID; 0.25 μm film thickness). Carrier gas was hydrogen, and the flow was set to a constant velocity of 40 cm sec⁻¹. The temperature was raised, following an isothermal hold at 50° C for 2 min, to 150° C at 20° C min⁻¹, then to 220° C at 3° C min⁻¹, followed by an increase to 340° C at 25° C min⁻¹ and a hold time of 5 min. Fatty acids were identified on an Agilent 6890 GC, fitted with an identical GC column, connected to an Agilent 5973 Mass Selective Detector. Representative fatty acid samples were also derivatised to produce fatty acid picolinyl esters following the method of Christie (1998); GC-MS analysis of these derivatives allows the determination of the positions of double bonds in the fatty acid chain.

GC-C-IRMS analysis of FAMEs was carried out on a Micromass Isoprime isotope ratio mass spectrometer connected to an Agilent 6890 GC via a combustion interface (630 mm x 0.3 mm i.d. containing a copper oxide/platinum catalyst, 850° C). Reference gas CO₂ of known $\delta^{13}\text{C}$ value was introduced at the start and end of each analytical run, and the performance of the IRMS checked with FAME standards of known $\delta^{13}\text{C}$ values

A.2. Cumulative rainfall lags and soil N₂O fluxes

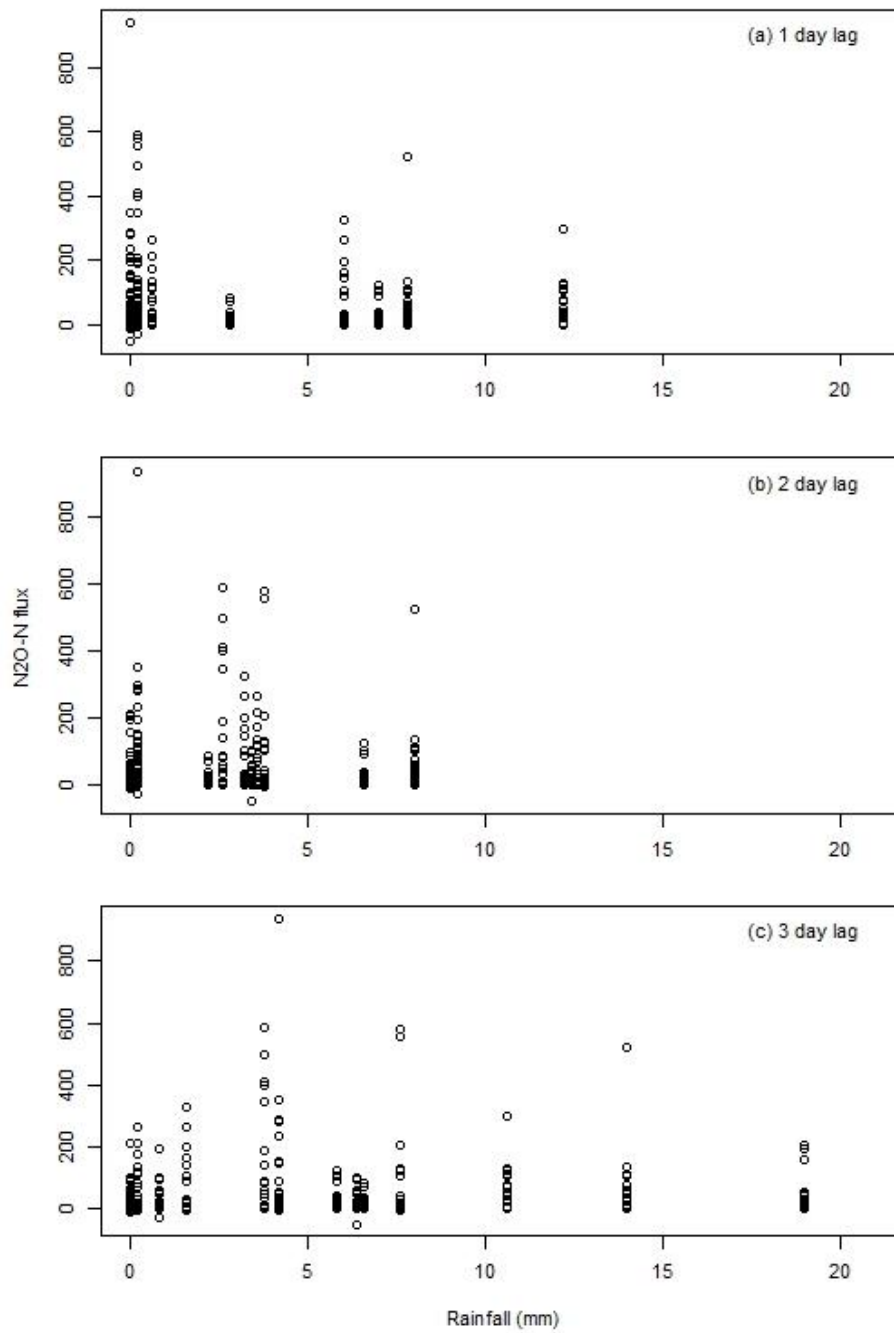


Figure 7.1 Relationships between cumulative rainfall lags (1 day, 2 days and 3 days before sampling) and soil N₂O flux.

A.3. Soil CO₂ efflux data for habitat, microtopography and water table over time

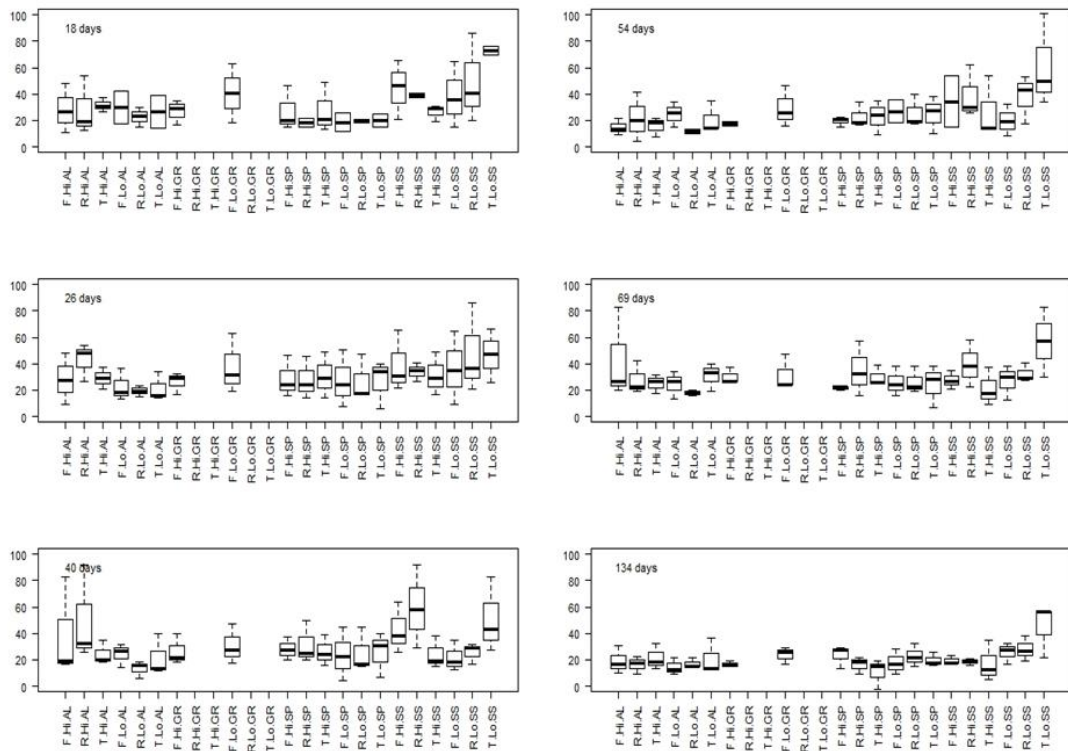


Figure 7.2 Soil CO₂ fluxes for all habitats, microtopographies and water table treatments over 134 incubation period. Microtopographies; (F) Flat, (R) Ridge and (T) Trough (Furrow). (LO) Low water table treatment 27 cm below surface and (Hi) High water table treatment 3 cm below surface. Habitats; (AL) Common alder, (GR) Grassland control (note only topography in grassland is (F) Flat), (SP) Scots pine and (SS) Sitka spruce. Error bars represent standard error. n = 3.

A.4. Soil CH₄ fluxes data for habitat, microtopography and water table over time

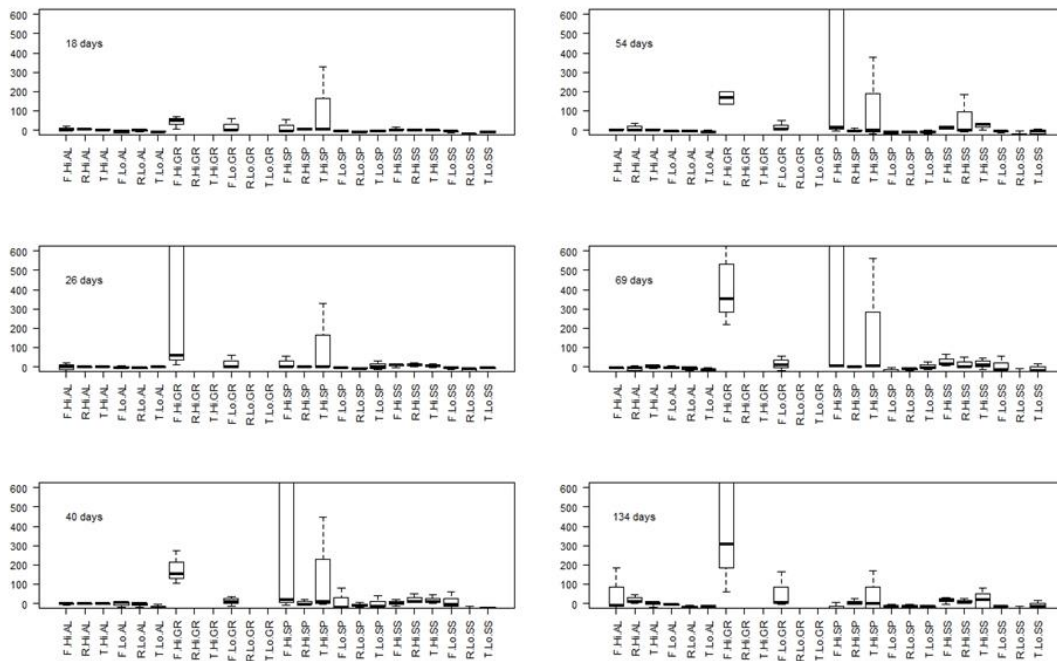


Figure 7.3 Soil CH₄ fluxes for all habitats, microtopographies and water table treatments over the 134 day incubation period. Microtopographies; (F) Flat, (R) Ridge and (T) Trough (Furrow). (Lo) Low water table treatment 27 cm below surface and (Hi) High water table treatment 3 cm below surface. Habitats; (AL) Common alder, (GR) Grassland control (note only topography in grassland is (F) Flat), (SP) Scots pine and (SS) Sitka spruce. Error bars represent standard error. n = 3.

A.5. Soil N₂O fluxes data for habitat, microtopography and water table over time

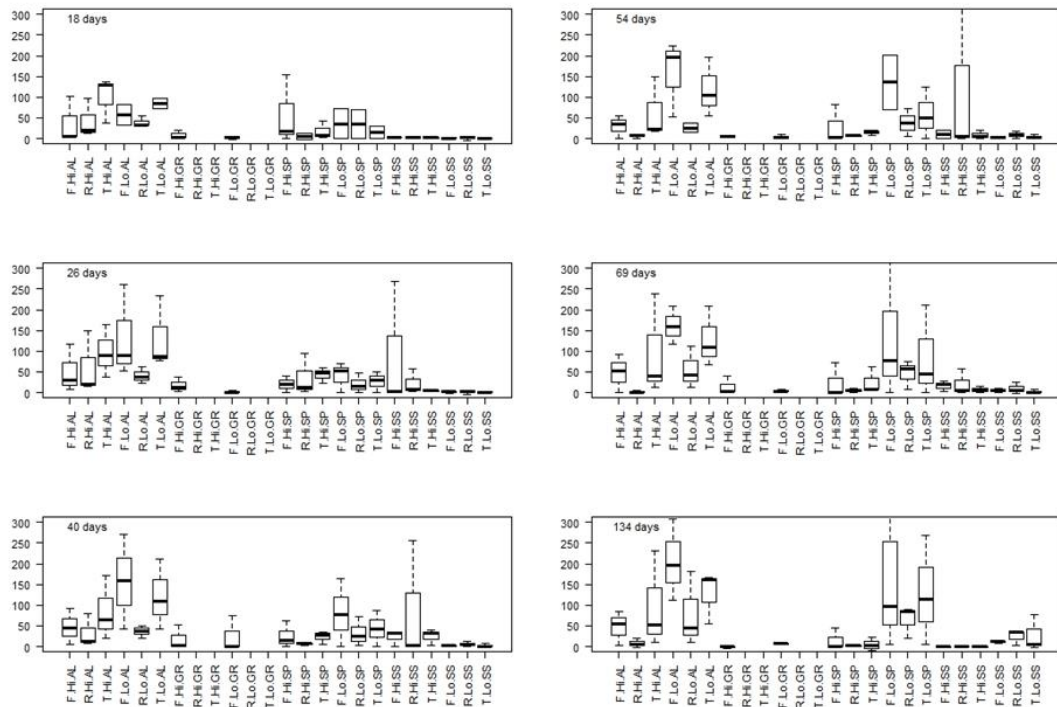


Figure 7.5 Soil N₂O fluxes for all habitats, microtopographies and water table treatments over 134 incubation period. Microtopographies; (F) Flat, (R) Ridge and (T) Trough (Furrow). (LO) Low water table treatment 27 cm below surface and (Hi) High water table treatment 3 cm below surface. Habitats; (AL) Common alder, (GR) Grassland control (note only topography in grassland is (F) Flat), (SP) Scots pine and (SS) Sitka spruce. Error bars represent standard error. n = 3.