Soil Greenhouse Gas Emissions and Soil C Dynamics in Bioenergy Crops

A thesis presented for the degree of Doctor of Philosophy at the University of Aberdeen

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March 2012

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Summary

Second generation bioenergy crops short rotation coppice (SRC) willow and *Miscanthus x giganteus* are the two main bioenergy crops grown in the UK. The first aim of this research was to quantify the in *situ* soil greenhouse gas (GHG) budget and to establish the drivers of these GHG fluxes for SRC willow and *Miscanthus*. The second aim of this research was to provide a more in-depth understanding of C cycling under *Miscanthus* i.e. litter and roots through two field experiments. The main findings were:

- The results from this work confirmed minimal emissions of CH_4 and N_2O from soil in second generation crops (non-food crops), SRC willow and *Miscanthus*.
- CO_2 flux was found to be the major efflux from soils in both crops and showed a positive correlation with temperature and showed a negative correlation with soil moisture content.
- The majority of total CO_2 flux from the soil surface under *Miscanthus* was from underground processes, with little contribution from aboveground litter decomposition to total flux.
- Litter played an important part in providing nutrients to the soil, which is vital in these crops since they are not fertilised.
- The high C:N ratio of *Miscanthus* litter and the high lignin content of SRC willow, resulted in an accumulation of litter on the soil surface and so may promote long-term C sequestration.
- Overall, the results from this work, combined with other literature would suggest that these crops offer advantages to first generation crops but more field-based studies are required to be able to say if these crops can offer large-scale GHG savings needed from this renewable energy source.

Author's Declaration

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

Signed:  Date: 31/03/12

Acknowledgements

Firstly, I would like to thank my supervisors, Dr Niall McNamara, Dr Jon Finch and Prof. Pete Smith for all the help and support they have given me over the course of my PhD. I would especially like to thank Niall, from which I have learnt a great deal and who has been so readily available when I needed help and advice.

I would like to thank the staff at Lancaster CEH who have been so friendly and helpful throughout my PhD. I would like to thank all those in Nutrients, especially Phil Rowland, Clive Woods, Kathryn Lehto and Jan Poskitt who gave me advice and helped with the analysis of many soil and litter samples. I am very grateful to Andy Stott and Helen Grant in SIF, who analysed hundreds of gas and bulk samples for ^{13}C. I thank Bev Dodd for allowing use of the vacuum pump for DOC analysis and Peter Henrys who gave me guidance on statistics. Thanks to my friends at CEH and Lancaster University; Aimee, Kathryn, Sean, Mike, Sue, Q, Sarah, Harriett, Kim, Rachel, Elaine, Rebecca, Ellie, Hayley, Andy, Simon and Alona; for keeping sane through the difficult times, helping out whenever they could and for the coffee breaks!

I am indebted to everyone who helped me with fieldwork, since without them I would not have been able to achieve half as much as I did. I would especially like to thank those who helped with the long, and in some cases dark days (literally) of trenching for the manipulation experiment; Niall McNamara, Simon Oakley, Nick Ostle, Mike Whitfield, Sean Case, Laura Barringer and Nicola Thompson.

Getting to this stage would not have been possible without the continued support and encouragement from my parents Ann and Stewart, to which I will always be grateful. This thesis is in part for them as it is for me.

Last, but by no means least, I would like to thank Paul, for all his love, support and understanding he has shown me during my PhD. I would like to thank him for the occasional help with R graphics and the cocktails! I owe him a great deal, especially for his patience during the write-up.

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Abbreviations

AIC	Akaike Information Criterion
ANOVA	Analysis of variance
BD	Bulk Density
C	Carbon
CH_4	Methane
Cl	Chlorine
CO_2	Carbon Dioxide
CO_2 eq.	Carbon Dioxide equivalent
DOC	Dissolve Organic Carbon
ECD	Electron Capture Detector
FID	Flame ionisation Detector
GC	Gas Chromatograph
GHG	Greenhouse Gas
GPP	Gross Primary Productivity
GLME	Generalised Linear Mixed Effects (model)
GS	Growing Season
GWP	Global Warming Potential
ha	hectare
HFA	Home Field Advantage
IRGA	Infra-red Gas Analyser
k	Decomposition Constant
K	Potassium
KCl	Potassium Chloride
LCA	Life Cycle Analysis
LFP	Litterfall Period
LME	Linear Mixed Effects (model)
N	Nitrogen
N_2O	Nitrous oxide
NEE	Net Ecosystem Exchange
NEP	Net Ecosystem Production
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NPP	Net Primary Production
P	Phosphorus

R_a	Aboveground Plant Respiration
R_b	Belowground Plant Respiration
R_L	Aboveground litter decomposition
R_m	Microbial Respiration
R_R	Rhizosphere Respiration
R_{SOM}	Soil Organic Matter Decomposition
R_T	Total Soil Respiration
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SRC	Short Rotation Coppice
t	tonne
WFPS	Water-filled Pore Space

Chapter 1

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1. General Introduction

1.1 Climate Change

The natural greenhouse effect of Earth's atmosphere is what allows us to be able to inhabit it. Under the greenhouse effect the suns energy is absorbed by the land, oceans and atmosphere with approximately one third of the energy being re-radiated back into space (Trenberth *et al*., 2009). Without atmospheric greenhouse gases (GHGs) such as water vapour, carbon dioxide (CO_2) and ozone along with other trace gases such as methane (CH_4) and nitrous oxide (N_2O), the earth would be too cold for human habitation. Through anthropogenic activities such as fossil fuel burning, land-use and agriculture, a rise in atmospheric GHGs has resulted in the net effect of global warming through increased radiative forcing (IPCC, 2007a). Since pre-industrial times atmospheric concentrations of CO_2, CH_4 and N_2O have risen from 280 ppm to 379 ppm, from 715 ppb to 1774 ppb and from 270 ppb to 319 ppb in 2005, respectively (IPCC, 2007a). 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 emissions compared to CO_2, their GWP are 298 and 25 times greater than a unit of CO_2.

1.2 GHG Emissions from Soils

At the global scale, soils are important sources of the three major radiatively forcing GHGs (Smith *et al*., 2007). Both natural and anthropogenic processes influence net emissions of these gases from soils. The contribution of soils to the global GHG

budget is increasing through intensification of agricultural practices and through conversion of natural systems to agricultural systems. It is recognised that soil sustainable management practices are important for GHG mitigation with European agricultural soils making up more than half the agricultural sector GHG emissions (UNFCCC, 2011). Trying to quantify, predict and understand GHG emissions from agricultural soils is complex due to the many underlying processes that can influence fluxes of CO_2, CH_4 and N_2O. The common factors that influence fluxes from soils are temperature, soil moisture, water-filled pore space (WFPS), soil type etc but agricultural soils also have several management practices that can also affect GHG fluxes, including additions of organic and inorganic fertiliser, tillage, liming and compaction from machinery (Li, 2000; Malhi *et al*., 2006; Malhi & Lemke, 2007).

1.2.1 Ecosystem CO_2 Exchanges Including Soil Respiration

CO_2 is removed from the atmosphere by plants through the process of photosynthesis but is returned to the atmosphere through a variety processes, collectively known as ecosystem respiration (Figure 1.1). The uptake of CO_2 by plants is known as gross primary productivity (GPP) and about half of this is returned to the atmosphere through respiration and the remainder making up net primary production (NPP). NPP is the total production of biomass (above and belowground) and dead organic material in a year. Net ecosystem production (NEP) is equivalent to the net carbon (C) stock change in an ecosystem and is NPP minus losses from heterotrophic respiration (microbial decomposition of soil organic matter (SOM) and aboveground litter). Net ecosystem exchange (NEE) is the difference between photosynthesis and ecosystem respiration (R_e) (Luo & Zhou, 2006).

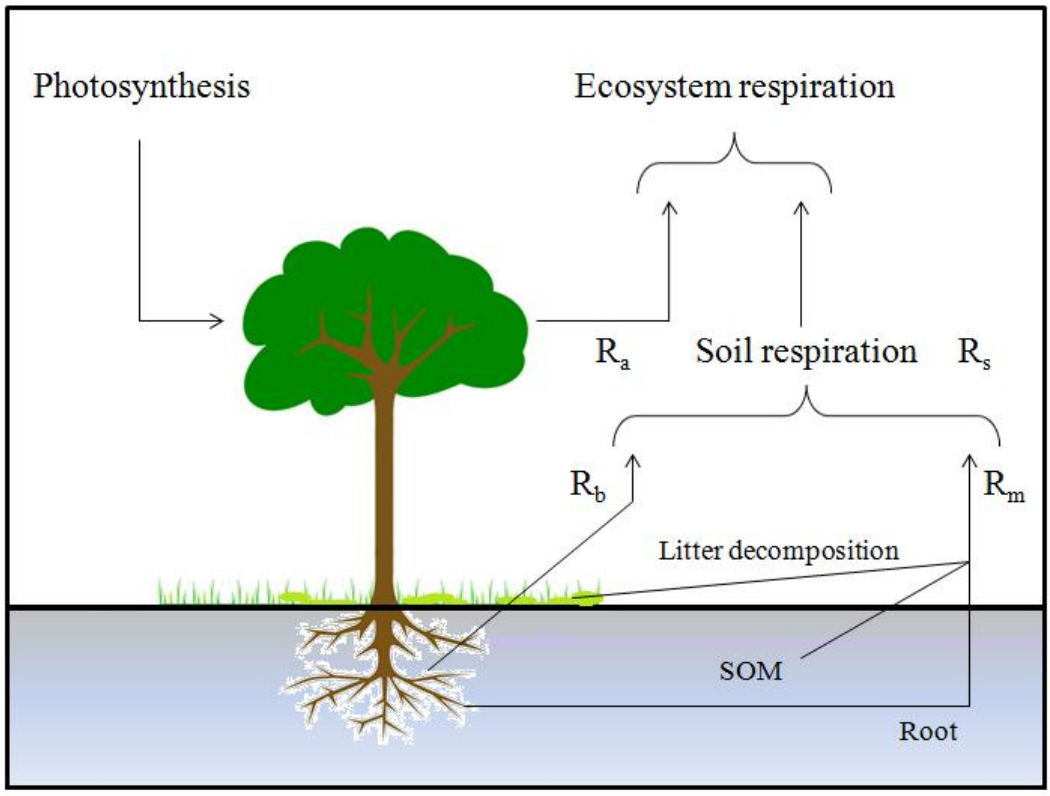

Figure 1.1 – Schematic diagram of ecosystem C processes. R_a is aboveground plant respiration, R_b is belowground plant respiration (root), R_m is microbial respiration (adapted from Luo & Zhou, 2006).

On a global scale, soil respiration is estimated to be in the range of 79 to 82 Gt C (Raich *et al*., 2002), making it the second largest C flux after photosynthesis and a key component of the global C balance (Schimel, 1995). As can be seen in Figure 1.1, soil respiration is contributed to by different processes and is generally separated into heterotrophic and autotrophic respiration. Autotrophic respiration from above (R_a) and below (R_b) living biomass is controlled by the amount of biomass, the nutrient content of biomass, the supply of sugars from photosynthesis and temperature (Ryan *et al*., 1997). In general it can be assumed that aboveground respiration is largely autotrophic but belowground autotrophic respiration often combined with heterotrophic respiration from root exudates. This is commonly called rhizosphere respiration and

although by definition this is heterotrophic respiration, it is often conceptually combined with autotrophic respiration (Trumbore, 2006).

Heterotrophic respiration is largely controlled by factors that affect microbial respiration such as temperature, soil moisture and the quality of the substrate being decomposed (Lloyd & Taylor, 1994; Davidson *et al*., 2002; Trumbore, 2006). The latter can often have large controls on the rate of decomposition and so the rate of CO_2 efflux. Although not universal, there are some general indices for the rate of decomposition. These include reducing decomposition with increasing carbon:nitrogen (C:N) ratio, lignin content and in some cases lignin:N ratio.

1.2.2 CH_4 Fluxes in Soils

CH_4 is produced in soils by methanogenic microbes during the breakdown of organic material under anaerobic conditions and accounts for more than a third of all CH_4 emissions (Smith & Conen, 2004). Net CH_4 emissions are commonly associated with wet habitats such as rice field and peatlands. Soils can also act as a sink for CH_4, through the oxidation of CH_4 in the soil by methanotrophic bacteria under aerobic conditions. Forest soils are known to have the highest oxidation rates, with reduced rates found in agricultural soils due to the disturbance from agricultural practices such as harvest and tillage and N fertiliser addition (Hütsch, 2001; Smith *et al*., 2000). The latter is known to negatively impact methanotrophic microbial communities through enzyme inhibition and so reduces the rate of oxidation in soils (Hütsch, 2001). CH_4 oxidation is largely controlled by soil moisture content and with increases in soil WFPS this generally reduces CH_4 oxidation by changing the diffusivity of the soil (Smith *et al*., 2003). The addition of crop residues to agricultural soils has also been

shown to effect CH_4 emissions through inhibiting CH_4 oxidation (Boeckx & van Cleemput, 1995).

1.2.3. N_2O Fluxes in Soils

Soils are a major source of atmospheric N_2O emissions and occur from both natural and agricultural sources (IPCC, 2007b). N_2O in soils is produced from the two contrasting microbial processes of nitrification and denitrification. Nitrification is an aerobic process and involves oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-). When the concentration of oxygen is limited nitrifying bacteria can use NO_2^- and reduce it to NO and N_2O (Smith *et al*., 2003). Dentrification is an anaerobic process and involves the reduction of NO_3^- to N_2O (and N_2). The largest emissions of N_2O are generally linked to dentrification but conditions of nitrification are more common so these fluxes are not trivial (Skiba & Smith, 2000). The main factors in controlling N_2O fluxes in soils are soil water content (through changing soil aeration) and N supply, although temperature also influences fluxes (Skiba & Smith, 2000; Smith *et al*., 2003). The highest N_2O emissions have been shown to occur with increasing WFPS especially from 70 to 90% (Dobbie *et al*., 1999). N_2O emissions are generally minimal in unfertilised soils, but in agricultural systems where N fertiliser in added, this can increase N_2O emissions rapidly (Dobbie *et al*., 1999; Skiba & Smith, 2000).

1.3 Bioenergy

Bioenergy is part of a suite of renewable energies that have become scientifically and politically important to combat the combined problems of climate change and energy security. Governments worldwide are considering using this and other renewable

energy sources as an alternative to coal, oil and gas, to reduce their national GHG emissions. There are also associated benefits in terms of energy security with supply of fossil fuels set to last just 118, 46 and 59 years respectively (BP, 2011). The use of bioenergy has become so important over the past decade that it is now part of legislation worldwide (National People's Congress (China), 2005; United States Congress, 2007, 2008; European Parliament, 2009). The UK aims to increase the amount of energy produced from bioenergy from 2% to 6% by the year 2020 (IEA energy statistic, 2007) to help achieve the ambitious target of reducing overall GHG emissions by 34% by 2020 from the 1990 baseline (Climate Change Act, 2008; The UK Renewable Energy Strategy, 2009; Energy Act, 2011).

Bioenergy is defined as the production of renewable energy from biological sources and is currently used in two main areas; power generation (electricity and combined heat and power) and liquid transport fuels (Karp & Shield, 2008). Although these processes also release CO_2 to the atmosphere, this is CO_2 that has been recently fixed by plants (via photosynthesis) from the atmosphere as opposed to being from stable fossil fuel stores. Since 1960, world energy consumption tripled, rising much faster than the population (Hein, 2005). Bioenergy contributes approximately 46 EJ y^{-1} (equivalent to 13.4%) to the overall worldwide primary energy supply, but it has been proposed that the contribution could be as much 400 EJ yr^{-1} by 2050 (Junginger *et al*., 2006). This depends on land availability and that the yields of bioenergy crops can be sustained.

In the UK, bioenergy is primarily used either for co-firing in electricity production or for local combined heat and power but with recent advances in lignoncellulose conversion technologies, some bioenergy crops could potentially be converted to transport fuels. It is estimated that the increase in energy from biomass crops will lead

to approximately 350,000 ha (hectares) of land being made available for growing bioenergy crops by 2020 (Department of Trade and Industry, 2007), bringing the total land available for biofuels and bioenergy crops in the UK to around 1 M ha (17% of arable land) (DEFRA, 2007).

Although there is great potential for bioenergy to reduce GHGs as compared to fossil fuels, it is not a simple issue. In order to assess whether bioenergy crops are truly going to offer the GHG savings that are needed, the whole bioenergy supply chain must be included in any GHG gas assessment. This means the incorporation of all processes that could emit GHG emissions, which could include anything from the fuel used in transporting the initial plantlets to the field, to soil processes resulting in GHG emissions under the crops. It is often the latter which is frequently lacking from any GHG assessment but is highly important (Whitaker *et al*., 2010; Nair *et al*., 2012).

1.3.1 First and Second Generation Bioenergy Crops

The vast majority of first generation bioenergy crops are used for the production of liquid fuel, but these are crops that are also used for food (such as wheat and maize), and have been considered to be unsustainable (Naik *et al*., 2010) due to the competition of land with food crops, high input requirements (e.g. N fertiliser) and negative effects on biodiversity. Competition with food crops for land has seen concerns raised over food security, due to the increased need for feedstock, especially from wheat and maize. Some studies have also linked this increase in first generation crop production to an increase in global food prices (Mitchell, 2008; Baier *et al*., 2009), but this is also confounded by other factors such as poor global wheat harvests and higher oil prices (Rosegrant, 2008). These crops also have high input requirements such as fertiliser, which may see high N_2O emissions, as a result of

fertiliser additions, offset any CO_2 savings due to the high GWP of this GHG (Smeets *et al*., 2009). The negative effects on natural biodiversity have been associated with land-use change from natural habitats to first generation crops (Dornburg *et al*., 2008). Second generation perennial bioenergy crops offer an alternative to first generation and alleviate many of the problems mentioned above (Havlík *et al*., 2011; Tan *et al*., 2008). Second generation crops are grown solely for the purpose of energy production and are not food crops. They can be grown on marginal or degraded land that is not suitable for food crops resulting in less competition for land. Second generation crops have been shown to have positive effects on biodiversity compared to first generation crops and annual crops (Rowe *et al*., 2009). This is likely to be due to the perennial natural of these crops, and in the case of Short Rotation Coppice (SRC) willow it is harvested on a 2-5 year cycle, providing a longer-term habitat than say annual crops (e.g. wheat). Second generations crops generally require fewer inputs from fertiliser and herbicides, cutting the management intensity and potentially reducing GHG emissions, especially of N_2O, which is a major sustainability issue associated with first generation crops. In the UK, the two main second generation crops are the perennial C_3 species willow (Salix spp.) and the C_4 energy grass *Miscanthus* (*Miscanthus x giganteus*).

1.3.2 Short Rotation Coppice (SRC) Willow

Willow has been used for centuries for a number of uses including basket making and healing (what we now call aspirin) but it was during the oil crisis in the 1970s that new interest in willow as a bioenergy crop came about (Karp *et al*., 2011). Willow showed promise as a bioenergy crop as it is suited to the UK's climate and soil conditions, easy to propagate, has a broad genetic base, has vigorous regeneration

after coppicing and high biomass production in SRC cycles (Grogan & Matthews, 2002; Keoleian & Volk, 2005). There are about 330-500 species of *Salix* but the main one used for the parent stock of most SRC willow varieties is *Salix viminalis* (DEFRA, 2004). A number of different varieties of SRC willow are usually planted within a plantation to help prevent spread of disease such as *Melampsora* rust and pests such as willow beetle (DEFRA, 2004). SRC willow is usually planted in spring either as cuttings or rods with around 15,000 stools ha^{-1} and is harvested approximately every three years, remaining viable for up to 30 years before replanting becomes necessary (DEFRA, 2004). The high CO_2-exchange rates, light-use efficiencies, photosynthetic capacities (Karp & Shields, 2008) can result in high yields, typically between 7 and 12 oven dried tonnes ha^{-1} yr^{-1} (DEFRA, 2004) but has been shown to reach yields as high as 18 ha^{-1} in optimum soil conditions (Fischer *et al*., 2005). There are approximately 6400 ha of SRC willow planted in the UK for the purpose of bioenergy and can be used in dedicated biomass burners or for combined heat and power production (DEFRA, 2009; Rowe *et al*., 2009). The advantages of SRC willow is that it needs very few inputs, especially of N fertiliser, which can reduce emissions of N_2O, and can be grown on marginal land or contaminated land (Vervaeke *et al*., 2003) so that it need not compete for prime agricultural land, both reducing concerns about the sustainability of bioenergy crops.

1.3.3 Miscanthus x giganteus

Miscanthus is a genus of about 14-20 species of perennial rhizomatous C_4 grass native to tropical and subtropical regions of Asia and Africa. *Miscanthus* is only one of a few genus that posses a C_4 photosynthetic pathway that can naturally occur in temperature

climates. Plants with C_4 photosynthesis tend to have higher radiation, water and N efficiencies and usually produce higher yields than C_3 plants.

Miscanthus and was first introduced to Europe in 1935, where it was noted for its vigorous growth, and has since been extensively trialled in Europe (Lewandowski *et al*., 2000). *Miscanthus x giganteus* (hereafter *Miscanthus*) is the most commonly trialled genotype and is a naturally occurring hybrid of *Miscanthus sacchariflorus* and *Miscanthus sinensis* (Greef & Deuter, 1993; Heaton *et al*., 2010). Many of the trials in the 1990's were part of the *Miscanthus* Productivity Network, under the Agro-industry Research Programme which investigated biomass potential, propagation and establishment, management practices, harvest and handling of *Miscanthus* (Lewandowski *et al*., 2000; Jones & Walsh, 2001).

Due to *Miscanthus* being a sterile hybrid it is propagated vegetatively and is planted either by rhizome cutting or *in vitro* culture (Lewandowski *et al*., 2000). *Miscanthus* is generally not harvested in the first year due to low yields caused by the sensitivity of young plants to frost damage in the first winter. Frost damage usually only occurs in the first winter even if subsequent winters are much harsher (Lewandowski *et al*., 2000; Jones & Walsh, 2001; Clifton-Brown & Lewandowski, 2000a). *Miscanthus* is harvested annually (after the first year) and experimental trials in the UK have shown yields to be greater than 13 t ha^{-1} (Bullard *et al*., 1997) once the crop has become fully established (after 2-5 years), although in Europe, yields have reached a maximum of 38 t ha^{-1} (Danalatos *et al*., 2006). The harvest is generally delayed until early spring since several studies have shown that this improves the combustion qualities of the crop by reducing ash, potassium (K), chloride (Cl), nitrogen (N), and moisture (Lewandowski *et al*., 2003; Jorgensen *et al*., 1997). The delay in harvest can reduce

the yield but with an earlier harvest the crop has a higher mineral content that can reduce the quality of the crop for combustion.

The advantage of *Miscanthus* is that it has a very low nutrient requirement such that it needs little or no additions of fertiliser, reducing management costs and potentially reducing N_2O emissions (Heaton *et al*., 2004; Rowe *et al*., 2009; Cadoux *et al*., 2012). This low N demand is explained through several reasons. Firstly, at the end of the growing season all nutrients are translocated to the rhizome for use during the following years' growth (Beal & Long, 1997; Himken *et al*., 1997). Secondly, a delay in harvest until late spring, commonly March or April, allows for large amounts of litter to fall over the winter period returning nutrients to the soil. Finally, several studies have shown that *Miscanthus* can fix N through free-living N fixing bacteria, reducing the need for fertiliser (Eckert *et al*., 2001; Miyamoto *et al*., 2004; Davis *et al*., 2010). N fertilisation experiments have shown that N has little or no effect on crop yield (Christian *et al*., 2008; Cosentino *et al*., 2007; Danalatos *et al*., 2007). Soil moisture content has a much bigger impact on yield and many seasonal differences in yield can be attributed to changes in soil moisture (Heaton *et al*., 2004; Price *et al*., 2004a). Generally, soils with a lower soil moisture content result in lower biomass yields, however, two studies from Cosentino *et al*., (2007) and Danalatos *et al*., (2007) still showed relatively high yields for *Miscanthus*, 14 t ha^{-1} and 28 t ha^{-1} respectively, even when grown in dry conditions.

1.3.4 Bioenergy Research – GHG emissions and C sequestration.

The majority of previous research associated with *Miscanthus* and SRC willow has been associated with improving propagation, establishment and yields. In recent years, given the concerns mentioned above, research questions are now arising which require

investigations of GHG emissions and C sequestration rates under these crops. To date, the latter has received more attention in both crops. It has been estimated that for SCR willow, C sequestration could be in the region of 0.22 to 1.6 t C ha^{-1} yr^{-1} over a 10 to 15 year period (Grigal & Berguson, 1998; Borzêcka-Walker *et al*., 2008). Jug *et al*., (1999) reported that the contribution to soil organic carbon (SOC) from SRC willow could be as much as 20% but other studies have found mixed results depending on previous land use and soil type (Grigal & Berguson, 1998; Hofmann-Schielle *et al*., 1999). *Miscanthus* has been estimated to sequester around 0.64 to 1.13 t C ha^{-1} y^{-1} (Matthews & Grogan, 2001; Borzêcka-Walker *et al*., 2008). Hansen *et al*., (2004) used natural abundance $^{13}C/^{12}C$ ratio differences between the C_4 crop and the historically C_3 planted soil to show after 9 and 16 years, 13% and 31% of the SOC at 0-20 cm, respectively, was derived from *Miscanthus*. The amount *Miscanthus* contributes to SOC has been found to be dependent on soil type and initial soil C content (Kahle *et al*., 2001).

To date, many of the published studies regarding GHG emissions are from estimates in life cycle analyses (LCA) (Hillier *et al*., 2009, Whitaker *et al*., 2010, Brandão *et al*., 2011) or modelled fluxes from soils using default values from the IPCC (Dondini *et al*., 2009). From the very limited field measurements available, it is suggested that there are negligible emissions of N_2O and CH_4 under SRC willow and *Miscanthus* compared to conventional crops (Drewer *et al*., 2011; Gauder *et al*., 2011) but a fuller assessment is required across multiple soil and climate types (Rowe *et al*., 2011). Further, it is now widely recognised that one of the values often missing from LCA are results from direct field scale measurements of GHGs (CO_2, CH_4 and N_2O) emitted from the soil and there has been a direct call for empirical data that is critical for model development and validation (Nair *et al*., 2012).

1.5 Research Aims and Objectives

The first aim of this research was to quantify the in *situ* soil GHG budget and to establish the drivers of these GHG fluxes for two UK second generation bioenergy crops, *Miscanthus* and SRC willow. The second aim of this research was to provide a more in-depth understanding of C cycling under *Miscanthus* i.e. litter and roots through two field experiments.

Chapters 2 and 3 focus on quantifying GHGs under SRC willow and *Miscanthus* respectively. The two main objectives were to describe the soil fluxes of CH_4, N_2O and CO_2 over two entire growing seasons and to investigate the environmental and soil chemical drivers that influence these GHG fluxes. An overall aim was to develop relationships that can be useful for predicting GHG fluxes as a function of these conditions. SRC willow was planted in 2000 and *Miscanthus* was planted in 2006 such that it was decided to not directly compare GHG fluxes between crops. Moreover, at the time of starting this study, October 2008, *Miscanthus* was in its third year of establishment while the SRC willow crop had already undergone 2 harvests.

In **Chapter 4**, a litter input and decomposition experiment under *Miscanthus* and SRC willow is presented. The aim of this research was to investigate litterfall dynamics, litter decomposition rates (litterbags) and to establish the main drivers (climate *versus* litter quality) behind decomposition through a reciprocal swap experiment.

The aim of **Chapter 5** was to establish the relative contribution of autotrophic respiration (defined here as root and rhizome plus associated rhizosphere organisms) and heterotrophic respiration to bulk soil respiration in *Miscanthus*. A litter and root (trenching) manipulation experiment was used, coupled with measurements of $^{13}CO_2$ to help identify the sources of soil respiration over a 22-month period, covering two growing seasons. It was hypothesised that root and SOM decomposition will

contribute the most to bulk soil respiration due to the extensive root system of *Miscanthus*.

In **Chapter 6**, the results from each chapter are reviewed with reference to the original aims and objective of the research. The main findings will be discussed within the context of bioenergy and what it has contributed to bioenergy research.

Chapter 2

Controls on greenhouse gas emissions (CO_2, CH_4 and N_2O) under the bioenergy crop short rotation coppice (SRC) willow

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Declaration of contribution

I declare that this chapter consists of original work undertaken by myself. The contribution of co-authors is as such. Sean Case and Niall McNamara assisted with fieldwork and Jon Finch provided continuous environmental data (temperature and soil moisture) from a nearby meteorological station, Niall McNamara, Jon Finch and Pete Smith provided PhD supervision and comments on this manuscript.

2.1 Abstract

A field study was conducted in a short rotation coppice (SRC) willow plantation in Lincolnshire, UK from October 2008 to November 2010 with the aim of measuring soil fluxes of CH_4, N_2O and CO_2 and to determine the main environmental and soil chemical drivers that influence these fluxes. Mean monthly CO_2 flux ranged from 10.4 to 243.4 mg CO_2-C m^{-2} h^{-1} and were clearly linked to seasonal changes with significant correlations found with temperature, soil moisture and soil C content, with temperature having the strongest control of CO_2 flux (r^2=0.86). Both N_2O and CH_4 fluxes were negligible, ranging from -9.7 to 32.2 µg CO_2-C m^{-2} h^{-1} for CH_4 and -6.3 to 4.8 µg N_2O-N m^{-2} h^{-1} for N_2O, with no significant relationships found with either environmental or soil chemical parameters. The overall soil GHG budget suggested that CO_2 was the primary efflux from the soil as management practices did not promote emission of either CH_4 or N_2O, the latter being a sustainability concern for certain bioenergy crops.

2.2 Introduction

Bioenergy is part of a suite of renewable energy sources that offers an alternative to fossil fuels, as reserves of conventional coal, oil and natural gas are set to last just 118, 46 and 59 years respectively (BP, 2011). Over the past few decades, the use of bioenergy sources for combined heat and power, and more recently a transport fuel, have increased and have now become incorporated into European (Renewable Energy Directive, 2009) (European Parliament, 2009) and international legislation (Energy Independence and Security Act of 2007, USA; Conservation and Energy Act of 2008, USA) (United States Congress, 2007, 2008). The advantage of bioenergy crops is that they are considered to have reduced greenhouse gas (GHG) emissions compared to fossil fuel based systems and can therefore contribute to reducing national emissions of GHG. In the UK, a commitment has been made to reduce overall GHG emissions by 34% by 2020 from the 1990 baseline, and bioenergy is an integral part of achieving this target (Climate Change Act, 2008; The UK Renewable Energy Strategy, 2009; Energy Act, 2011). It is estimated that approximately 350,000 ha (hectares) of land could be made available for growing bioenergy crops by 2020 (Department of Trade and Industry, 2007), bringing the total land available for biofuels and bioenergy crops in the UK to around 1 M ha (17% of arable land) (DEFRA, 2007).

One of the most promising bioenergy crops in the UK is the perennial short rotation coppice (SRC) willow, but there are currently only approximately 6000 ha planted in the UK (DEFRA, 2009). This crop has relatively high yields, requires very few inputs, especially nitrogen (N) fertiliser and can be grown on marginal land or contaminated land (Vervaeke *et al*., 2003) so that it need not compete for prime agricultural land. There are many species of SRC willow but the main one used for the parent stock of most SRC willow varieties is *Salix viminalis* (DEFRA, 2004). This fast growing wood

species has high carbon dioxide (CO_2)- exchange rates, light-use efficiencies, photosynthetic capacities (Karp & Shields, 2008) and high yields, typically between 7 and 12 oven dried tonnes (odt) ha^{-1} yr^{-1} (DEFRA, 2004). SRC willow is harvested approximately every three years and can remain viable for up to 30 years before replanting becomes necessary (DEFRA, 2004).

The extent of GHG saving by SRC willow or other energy crops is strongly influenced by the whole life cycle of biomass production, from growing the crop to harvest, processing and transportation of biomass feedstocks and products (Hillier *et al*., 2009). While mitigating CO_2 emissions through fossil fuel substitution is a primary benefit of using biomass for energy, it is now recognised that GHG emissions from this whole bioenergy supply chain, including non-CO_2 GHGs, must also be factored into any GHG assessment (Whitaker *et al*., 2010). This is because both methane (CH_4) and nitrous oxide (N_2O) can potentially significantly influence an overall GHG budget, due to their much higher global warming potential (GWP) (25 and 298 for CH_4 and N_2O respectively) (IPCC, 2007a). Further, it is now widely recognised that one of the values often missing from life cycle analyses (LCA) are results from direct field scale measurements of GHGs (CO_2, CH_4 and N_2O) emitted from the soil (Nair *et al*., 2012). Many of the published studies regarding GHG emissions are from estimates in LCAs (Hillier *et al*., 2009; Whitaker *et al*., 2010; Brandão *et al*., 2011) or modelled fluxes from soils using default values from the IPCC (Dondini *et al*., 2009). From the very limited field measurements available, it is suggested that there are negligible emissions of N_2O and CH_4 under SRC willow compared to conventional crops (Drewer *et al*., 2011; Gauder *et al*., 2011) but a fuller assessment is required across multiple soil and climate types (Rowe *et al*., 2011).

N_2O in soils is produced through the microbial processes of nitrification and denitrification under aerobic and anaerobic condition respectively. These processes need either ammonium (NH_4^+) or nitrate (NO_3^-) therefore N_2O production is highly influenced by N fertiliser application. In the absence of fertiliser additions, N_2O can also be released from the decomposition of organic material (litter), through either process depending on the aeration of the soil (Baggs *et al*., 2000). Since SRC willow receives minimal fertiliser additions and has less N demand than other bioenergy crops, for example maize, it is likely that SRC willow will have favourable climate impacts (Crutzen *et al*., 2008). Net CH_4 fluxes from the soil result from two microbial processes; methanogensis (CH_4 production) and methanotrophy (CH_4 oxidation), which generally occur in soils under anaerobic and aerobic conditions, respectively. Aerobic soils are generally thought to be net sinks for CH_4, however the relative strength of this sink is diminished in response to soil disturbance and nitrogen inputs (Smith *et al*., 2000; Hütsch, 2001). It is therefore possible that perennial non-food (second generation) bioenergy crops are more likely to favour methanotrophic populations than arable soils. Field CO_2 emissions are the net balance between carbon (C) fixation into plant biomass and losses through decomposition of plant biomass and soil and root respiration. Due to the minimal cultivation of perennial crops, decomposition is often slower than in arable crops, which is likely to result in lower CO_2 emissions. Overall, soils and vegetation play an important role in both releasing and consuming all three of these GHG gases. Over the long-term, net GHG emissions from the soil will be strongly influenced by factors such as crop type, management practices, soil pH and initial soil C content (Malhi *et al*., 2006). At hourly and daily time scales, the instantaneous GHG flux is regulated by more dynamic environmental variables such as the soil climate, temperature and moisture (Xu & Qi, 2001).

2. Controls on GHG emissions from SRC willow

The first objective of this study was to describe the soil fluxes of CH_4, N_2O and CO_2 over two entire growing seasons under a SRC willow plantation. The second objective of our study was to investigate the environmental and soil chemical drivers that influence GHG fluxes from under SRC Willow, with the aim of developing relationships that can be useful for the future modelling of GHG fluxes as a function of these conditions. Overall, we would like to see this information used to parameterise and/or validate LCAs and soil C models. Improving predictive models for soil C after land use change is required to ensure that the most sustainable pathways are chosen for future bioenergy expansion.

2.3 Materials and Methods

2.3.1 Study Site and Experimental Design

The field experiment was conducted in a SRC willow plantation on a commercial farm in Lincolnshire, UK. The underlying soil type is a fine loam over clay, with approximately 25, 29 and 49 % clay, sand and silt content, respectively. The mean annual precipitation was 605mm (30-year average 1980 to 2010) and the mean annual temperature was 9.9 °C (30-year average) (Table 2.1). The soil had a pH of 5.6 and a mean total C and N content of 1.81 % and 0.28% respectively, with further soil properties shown in Table 2.2.

The SRC willow was established in 2000 at a planting density of 15,000 stools ha^{-1} and covers approximately 9.44 ha. It was planted in twin rows, about 0.75 m apart and 1.5 m between each set of twin rows. Different varieties of SRC willow were planted to prevent disease spread across that plantation, with the most common variety being Tora. The crop was first coppiced in 2001 and then has been harvested, in 2004, 2007 and 2011, with yields of 20, 26 and 19 t ha^{-1} respectively. The growing season for SRC willow is typically between March and September. The land use prior to the establishment of the SRC willow was a crop rotation with wheat and oil seed rape. The plantation has received no fertiliser or herbicide during this study.

In October 2008, five randomly chosen sampling blocks were established in the SRC willow. The minimum distance between blocks was 27 m and the maximum distance between blocks was 189 m. From these blocks, a range of measurements were taken on a monthly basis until November 2010. Measurements included soil GHG measurements (CO_2, CH_4 and N_2O); soil solution chemistry (DOC, NH_4^+ and NO_3^-); and associated ancillary measurements of soil and air temperature and soil moisture.

2.3.2 GHG Measurements

CH_4 and N_2O measurements were based on the static chamber method described by Livingston & Hutchinson (1995) and CO_2 measurements were made with a dynamic system using an infra-red gas analyser (IRGA, EGM-4 PP Systems) directly connected to the static chamber. The static chamber method was adapted to include the use of a small fan and 'vent'. The fan was carefully designed to ensure a slight mixing of the chamber air only and the vent, which comprised of a Tedlar bag (SKC Ltd, UK) connected to the outside of the chamber using 4 mm gauge tubing (Nakano *et al*., 2004) was designed to compensate for pressure changes within the chamber. The static chambers were made from PVC (40 cm diameter, 20 cm in height) and were inserted approximately 3 cm into the soil. In 2009, one chamber was placed in the planted rows (hereafter IR) of SRC willow at each sampling block. For the 2010 season, an additional chamber was inserted between the rows (hereafter BR) of SRC willow at each sampling block and this was to evaluate if there were any differences in soil GHG emissions between IR and BR. All chambers remained in the soil for the duration of the study and were enclosed at the time of sampling with a reflective aluminium lid, which had rubber seal around the edge to prevent leakage.

Soil respiration using the IRGA were made prior to the CH_4 and N_2O measurements and took approximately three minutes to complete. For CH_4 and N_2O measurements, chambers were enclosed for 30 minutes with two 10 ml gas samples taken every 10 minutes (four samples per chamber). At the time of sampling, gas samples were transferred from the chamber headspace into a gas-tight 3 ml exetainer (Labco Ltd, UK) via needle and syringe inserted into the self-sealing septa in the chamber lid. All measurements (CO_2, CH_4 and N_2O) were taken between the hours of 10:30 and 14:30 on the day of sampling. Gas samples were analysed for CH_4 on a Perkin Elmer

Autosystem GC fitted with a FID and analysed for N_2O Perkin Elmer Autosystem XL GC fitted with an ECD (McNamara *et al*., 2008). All results were calibrated against certified gas standards comprising of 1.06 ppm CH_4 and 1.07 ppm N_2O in air (BOC, UK). Gas fluxes (CO_2, CH_4 and N_2O) were calculated from the change in chamber concentration, field air temperature and chamber volume and area measurements using the method in Holland *et al*., (1999).

A soil GHG budget depends on the net balance of CO_2, CH_4 and N_2O emissions during a defined period, e.g. annual or over the growing season. Annual and growing season fluxes were extrapolated from the mean monthly values and average values over the total period of measurement. To do this, soil fluxes of CH_4 and N_2O were converted to CO_2 equivalents (CO_2 eq.) based on their global warming potentials (GWP) of 25 and 298 respectively according to the 100-year time-frame (IPCC, 2007a).

2.3.3 Soil Sampling and Analysis

Fresh soil samples (0-15 cm depth, 5 cm diameter) were taken within a three-metre radius of each chamber on each monthly visit, after the soil GHG measurements. Samples were used to determine gravimetric moisture; water-filled pore space (WFPS); total C and N; inorganic N, ammonium ($NH4^+$-N) and nitrate ($NO3^-$-N) and soil extracted dissolves organic C (DOC). Gravimetric moisture was determined from a 10 g subsample placed in an oven at 105°C for 24 hours. WFPS was calculated using the gravimetric moisture, bulk density (BD) and the density of quartz (2.65 g cm^{-3}). Total C and N were determined from ground subsamples using a TruSpec CN analyser (LECO, UK). Inorganic N concentration was determined by KCl (6%) extraction. The extracts were analysed for $NH4^+$-N and $NO3^-$-N colourmetrically

using a AQ2 discrete analyser (Seal Analytical Ltd., UK). DOC was measured by adding 70 ml of distilled water to 10 g of soil and shaking it on an orbital shaker for 10 minutes (Harrison & Bardgett, 2003). The solution was filtered twice; firstly through a coarse Whatman No. 1 filter and secondly, under vacuum, through a 0.45µm cellulose nitrate filter paper (Whatman, UK) (Ward *et al*., 2007). DOC was determined using a Shimadzu 5000 TOC analyser.

2.3.4 Field Climatic Measures

Climatic conditions were noted at each sampling visit. This included measures of air and soil temperature using a Tiny Tag (view 2) temperature logger with integral stab probe for soil temperature (0-7 cm depth) (Gemini Data Loggers, UK) and measurement of volumetric soil moisture (0-6 cm) using a ML2x Theta Probe and Meter HH2 (Delta T Devices, UK) (hereafter Theta moisture). Theta moisture content was determined by taking the mean of three measurements taken from close to the chamber during gas measurements. Continuous measurements of precipitation (203 mm diameter automated tipping rain gauge, Rimco 8500) were made from an automatic meteorological station near to the SRC willow field.

2.3.5 Statistical Analysis

All statistical analysis was performed using R (version 2.14.0). Mean monthly values for each GHG, soil and meteorological variants were calculated and then monthly mean values over the 25-month period were tested for normality and transformed where appropriate. For CH_4 and N_2O, a constant of 20 and 5 was added respectively before analysis as these were equivalent to the lowest measured value of each gas. A Student's t-test was used to determine if there were any significant differences

between IR and BR CO_2 flux for each month. Pearson Product Moment Correlation was used to relate gas flux data to soil and meteorological data. Linear mixed effects models were used to determine the most influential soil and meteorological parameters on soil GHG fluxes. This was done by starting with a model with all soil and meteorological parameters included and then these were removed one by one starting with the least significant parameter until the model was left with only significant parameters. Since a) air and soil temperature and b) Theta and gravimetric moisture were likely to be highly correlated, these were not all included in the model together. Instead four different combinations were used with other soil and meteorological parameters; soil temperature and Theta moisture; soil temperature and gravimetric moisture; air temperature and Theta moisture; and air temperature and gravimetric moisture. The best model fit was chosen according to the Akaike Information Criterion (AIC) (the lower the value the better the model fit) and model graphical outputs. 'Date' was used as a random effect to account for repeated measures over time. The significance of all results was accepted when $p<0.05$.

2.4 Results

2.4.1 Field Climatic Measures

Air temperatures ranged from 0.3 to 30 °C, with the highest temperatures recorded in July 2009 and 2010 (Figure 2.1). Soil temperatures followed a similar pattern and ranged from 0 to 22.5°C. Both 2009 and 2010 had higher mean growing season temperatures than the 30-year average (Table 2.1) by at least 3.8 °C. The mean soil temperature for the growing season was the same in both years (13.1 °C). The mean annual precipitation for 2010 was higher than in 2009 and the 30-year average precipitation due to heavy precipitation in January 2009. The mean growing season precipitation was similar for 2009, 2010 and the 30-year average (Table 2.1).

Table 2.1 - Summary of climatic data for SRC willow, Lincoln, UK. Values for air and soil temperature and precipitation for 2009 and 2010 are an average of mean monthly (n=5) values. Annual values for 2010 temperature were not added since an incomplete year of measurements were made.

Year	Mean Air Temperature (°C)	Mean Soil Temperature (°C)	Precipitation (mm)
Annual			
30-year average†	9.9	-	605
2009	12.9	10.1	679
2010	-	-	545
Growing Season‡			
30-year average†	12.6	-	357
2009	16.4	13.1	326
2010	17.8	13.1	310

† 30-year average was from 1980 to 2010. Data from a local meteorological station.
‡ Growing season is define here as March to September.

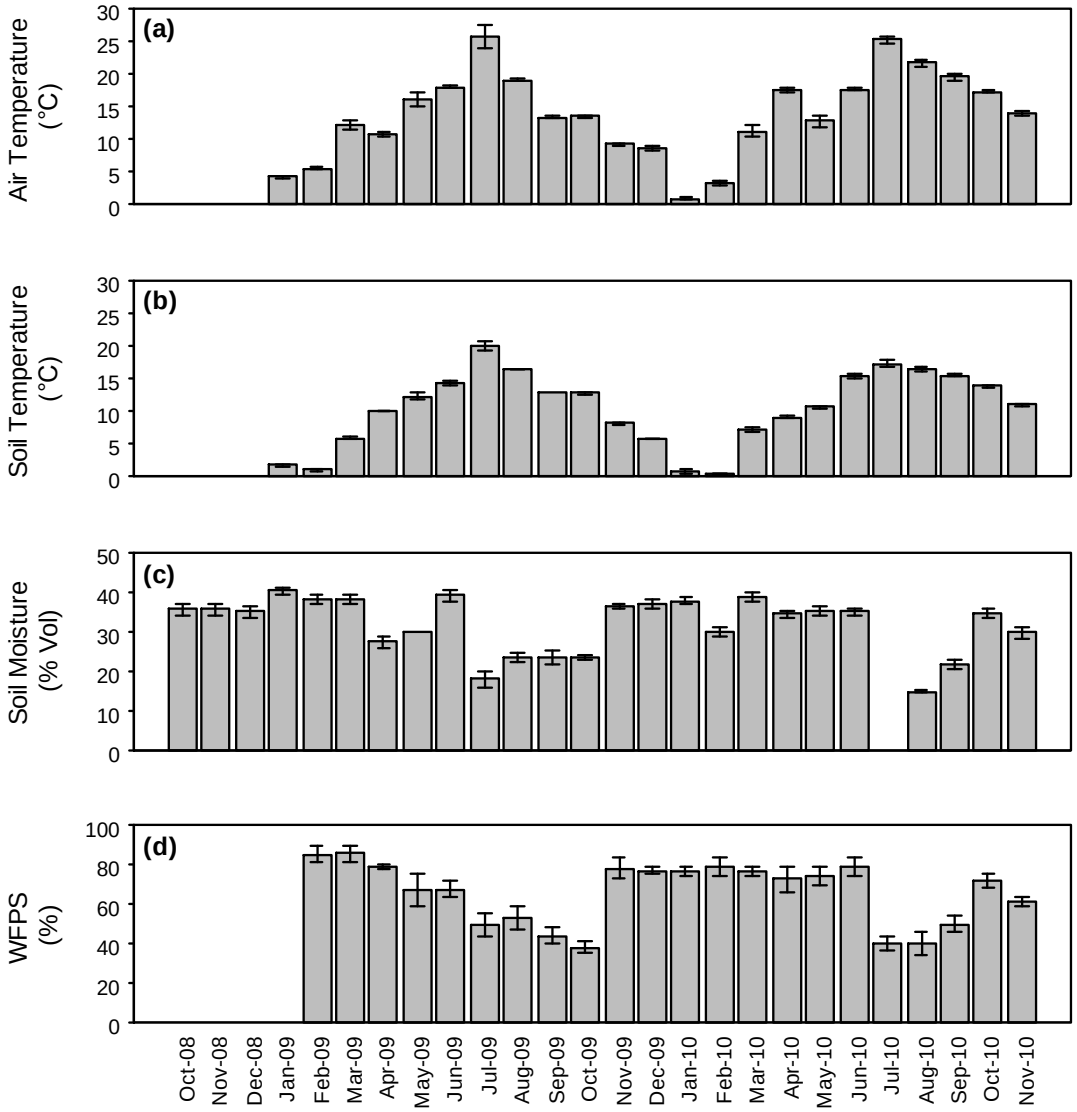

Figure 2.1 - Mean monthly values (n=5) of **a)** air temperature, **b)** soil temperature, **c)** Theta moisture (0-6 cm depth) and **d)** WFPS (0-15 cm depth) taken. Error bars represent standard error values.

2.4.2 Soil Physical and Chemical Properties

The soil BD, total C and N remained constant throughout the study (Table 2.2) and had a mean values from across the 25-month study period of 1.3 g cm^{-3}, 1.9% and 0.3%, respectively. Theta moisture was typically above 30% over the winter months and part of the early growing season (March to June) and generally fell below 30% during periods of high temperatures (July, August and September) (Figure 2.1). WFPS ranged from 25.8% to 98.8% over the duration of the study but was typically above 60% with the exception of months July, August and September in both years and Oct in 2009, which coincided with periods of high temperatures (Figure 2.1).

Table 2.2 - Summary of soil properties for SRC willow, Lincoln, UK. Soil properties were determined from soil cores (n=5, 0-15 cm depth) collected each month and values are an overall mean of mean monthly values ± standard error values.

Parameter	2009	2010
Bulk Density (g cm^{-3})	1.34 ± 0.02	1.33 ± 0.02
Total C (%)	1.83 ± 0.05	1.80 ± 0.07
Total N (%)	0.27 ± 0.004	0.30 ± 0.01
C:N Ratio	6.69	5.91

The soil samples collected at each monthly visit were used to determine DOC, NH_4^+ and NO_3^-. DOC values ranged from 34.3 to 160.5 µg g^{-1} dry weight of soil and no seasonal trend was observed (Figure 2.2). There were no significant correlations found with any meteorological or soil parameters. Soil NH_4^+ concentrations ranged from 1.0 to 10.7 mg kg^{-1} over the total study period. There was a tendency for there to be an increase in NH_4^+ concentration over the early growing season (February to May) in

both 2009 and 2010 and then a decline in concentration with the lowest concentrations detected after the growing season and over winter. However, no significant correlations were found with any other soil or meteorological parameters. NO_3^- concentrations were generally lower than that of NH_4^+ concentration and ranged from 0.1 to 7.5 mg kg^{-1}. There was no clear trend in the data and no significant correlations were found with soil or meteorological data.

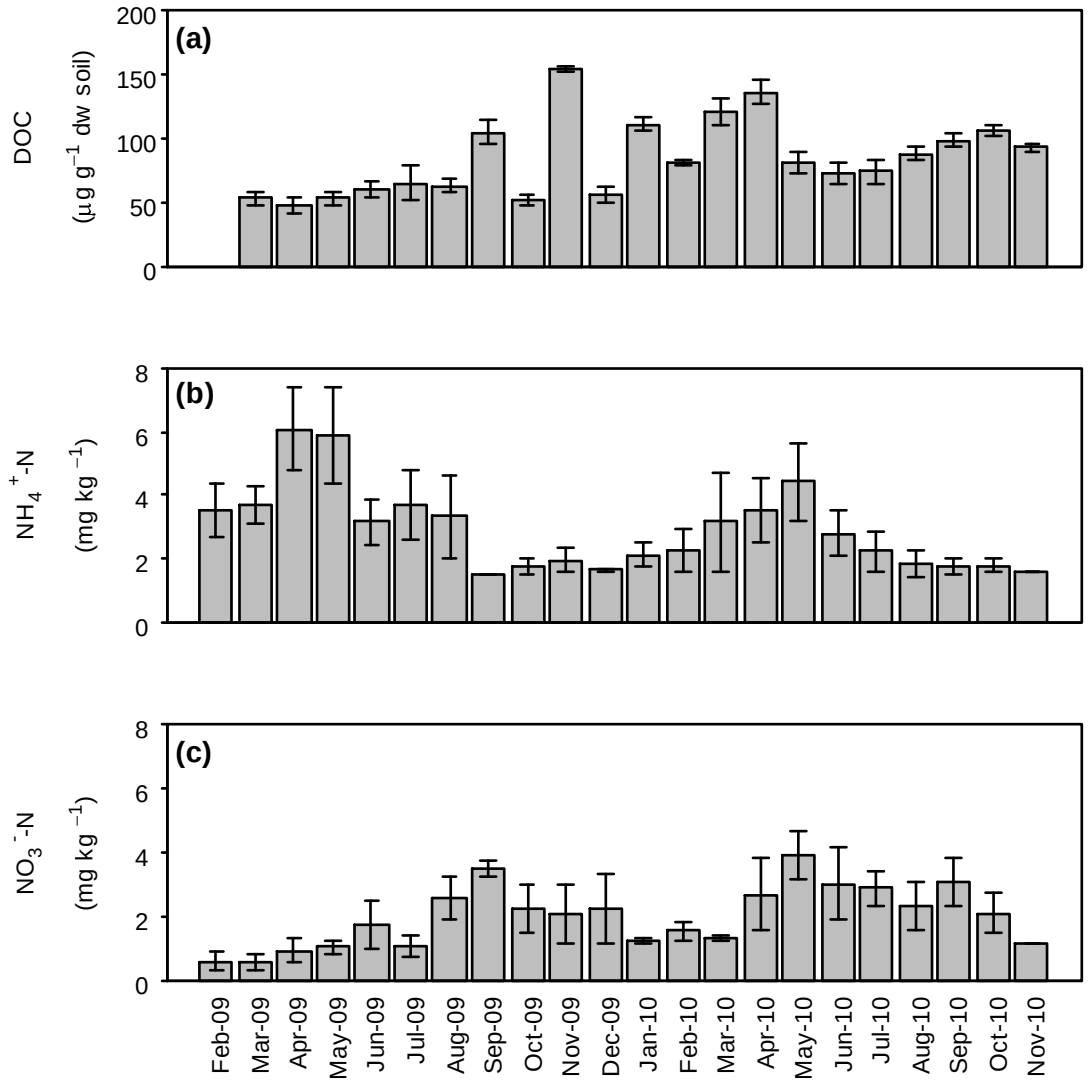

Figure 2.2 - Mean monthly values (n-5) for **a)** DOC, **b)** NH_4^+ and **c)** NO_3^-, extracted from soil cores (0-15 cm depth) taken at each sampling visit. Error bars represent standard error values.

2.4.3 Soil GHG Emissions

Soil respiration showed a strong seasonal pattern with fluxes in summer reaching highs of 394 mg CO_2-C m^{-2} h^{-1} (July 2009, individual data point) and winter fluxes being much lower, ranging between 0 and 30 mg CO_2-C m^{-2} h^{-1} (Figure 2.3a). There was little difference in CO_2 flux in between IR and BR, with only two months, June and October 2010, showing a significant difference ($p<0.05$) in measured fluxes (Figure 2.3d). Soil respiration was significantly correlated with several meteorological and soil parameters (Table 2.3).

Table 2.3 - The meteorological and soil properties that showed significant correlations with CO_2 flux. Values are the Pearson production-moment correlation coefficient (r) and the associated significance (p value). Levels of significance are $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) for F values at 1, 18 *df*.

Parameter	Air Temp	Soil Temp	Theta Moisture‡	Gravimetric Moisture†	WFPS	Total C
R	0.84	0.88	-0.60	-0.77	-0.69	0.53
F value	43.67 (***)	97.18 (***)	10.21 (**)	26.70 (***)	16.12 (***)	7.24 (*)

‡ 0-6 cm depth and † 0-15 cm depth

Temperature (air and soil) was found to have the strongest correlation (r=0.84 and r=0.88 respectively), followed by gravimetric moisture (r=-0.77), WFPS (r=-0.69), Theta moisture (r=-0.60) and total soil C (r=0.53). The strongest of these relationships are shown in Figure 2.4, with temperature explaining 86% of the variation and soil moisture and WFPS explaining about 50% of the variation. No significant correlations were found between CO_2 flux and DOC, NH_4^+ or NO_3^- (Table 2.3). The analysis using a mixed effects models showed that soil temperature produced the best model fit in estimating CO_2 flux ($p<0.0001$). Other model combinations suggested that WFPS and

gravimetric moisture content might also be important in CO_2 emissions but the model fit was less good and these models were not used.

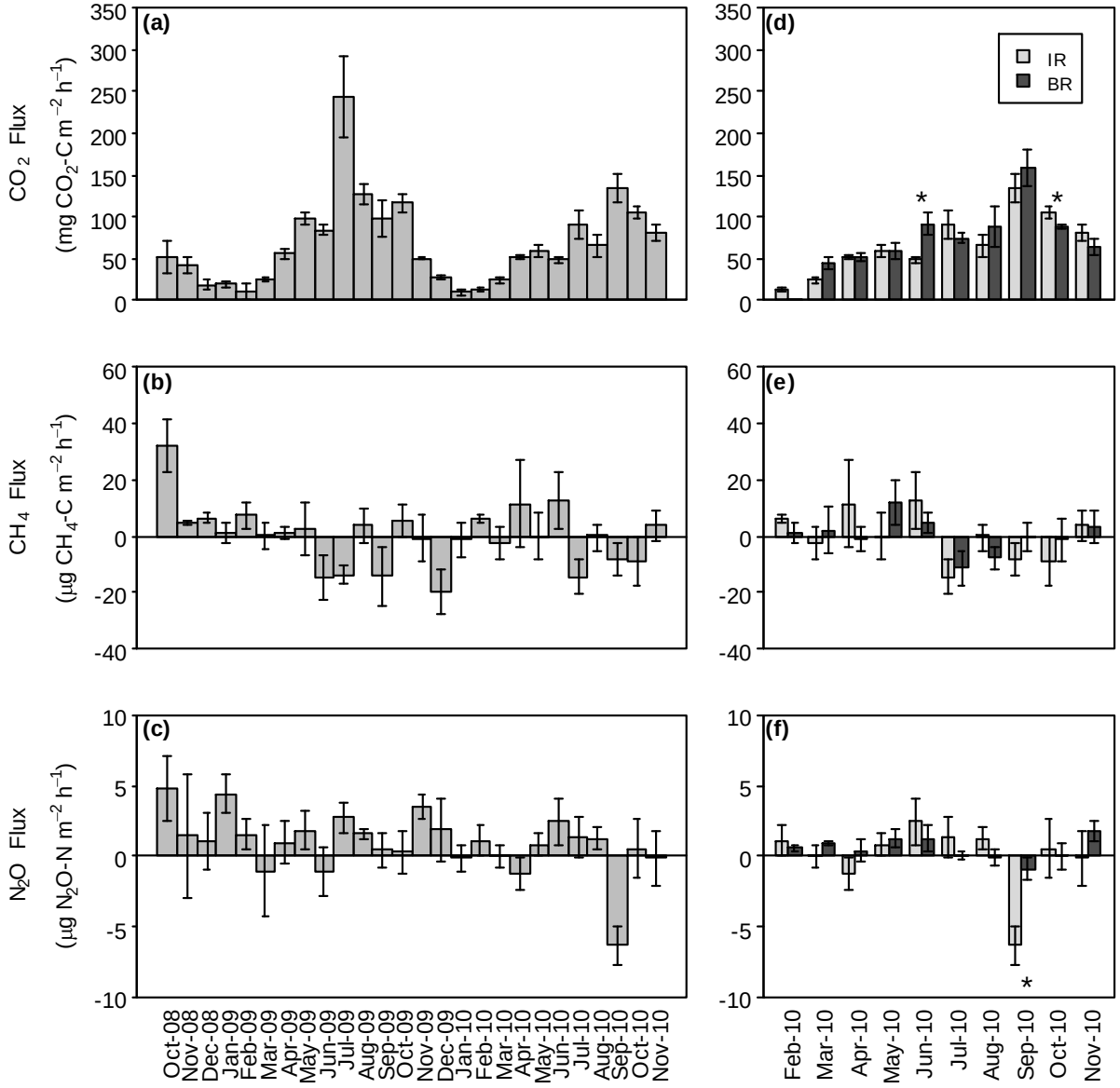

Figure 2.3 - Plots **a)**, **b)** and **c)** represent mean monthly (n=5) in row (IR) fluxes of CO_2, CH_4 and N_2O respectively from October 2008 to November 2010. Plots **d)**, **e)** and **f)** represent mean monthly (n=5) from IR and Between Row (BR) fluxes of CO_2, CH_4 and N_2O respectively from February 2010 and November 2010. Error bars represent standard error values.* indicates a significant difference between IR and BR fluxes. Significance accepted when $p<0.05$.

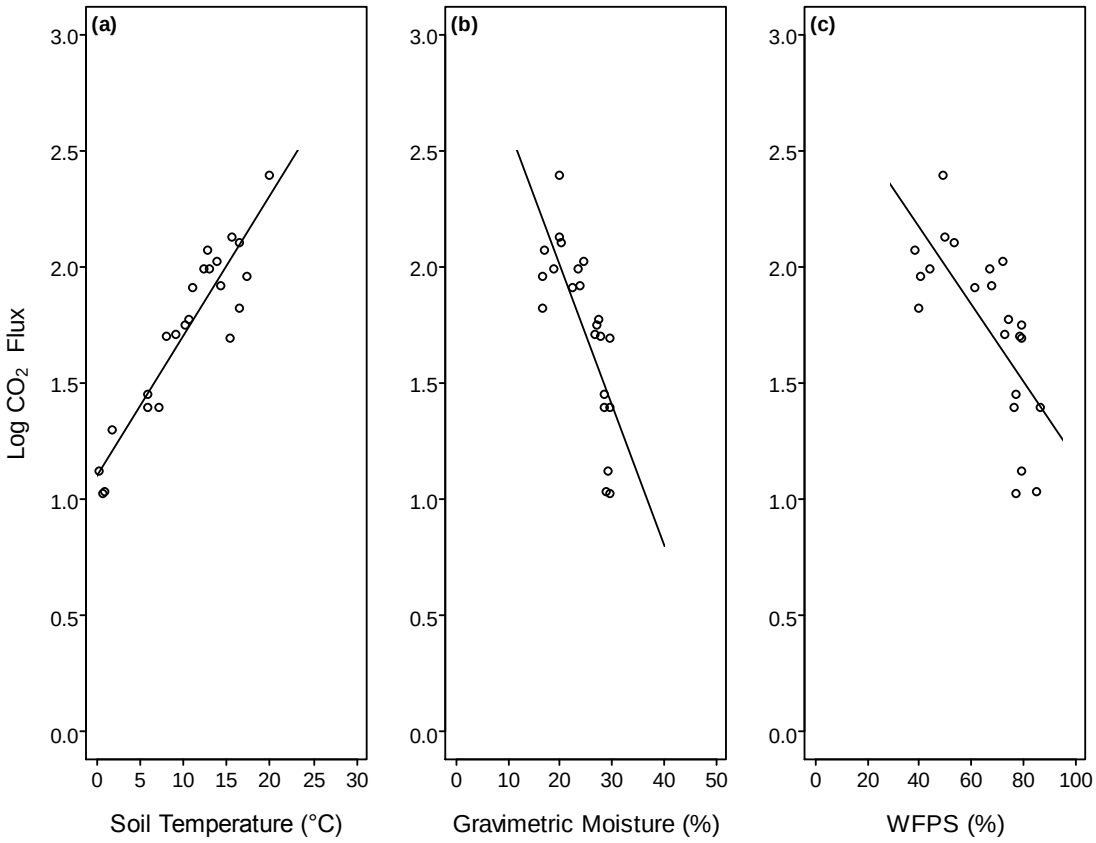

Figure 2.4 - Correlations between logged CO_2 flux and **a)** Soil temperature (0-7 cm depth), **b)** gravimetric moisture (0-15 cm, depth) and **c)** Water-filled pore space (WFPS) (0-15 cm, depth). Values are mean monthly values (n=5).

CH_4 fluxes varied between -45.6 and 50.2 µg CH_4-C m^{-2} h^{-1} (individual data) over the study period but showed no seasonal trend (Figure 2.3b) and no significant differences between fluxes measured in IR or BR were found (Figure 2.3e). Due to there being no overall trend to the flux, no significant correlations were found with any meteorological or soil parameters.

N_2O fluxes were negligible and varied between -9.0 and 9.9 µg CH_4-C m^{-2} h^{-1} (individual data). There appeared to be a tendency for N_2O fluxes to decrease over the winter months (Oct to Feb) and over the growing season (Mar to Sep) (Figure 2.3c) but it is difficult to determine if this is a 'real' trend due to the associated variability between samples. There was one month (September) that showed a significant difference between IR and BR fluxes (Figure 2.3f). No significant correlations were found with any meteorological or soil parameters. Mixed models found no significant parameters but suggested that total soil N, gravimetric moisture and WFPS may be important in influencing N_2O emissions.

2.4.4 Soil GHG Budget

A GWP approach was used to calculate a GHG budget for SRC willow (Table 2.4). It is clear from Table 2.4 that soil respiration is the main contributor to the overall soil GHG budget and contributions ranged from 596 to 916 g CO_2 eq. m^{-2} y^{-1}. SRC willow soils were shown to be a weak sink for CH_4 since for all periods shown (annual and growing season) CH_4 emissions were negative (Table 2.4). The GWP for CH_4 ranged from -0.06 to -1.09 g CO_2 eq. m^{-2} y^{-1}, over the growing seasons for 2009 and 2010 respectively. N_2O behaved differently between 2009 and 2010. The annual values of GHG for 2009 were 1.42 g m^{-2} y^{-1} with a positive GWP of 3.72 g CO_2 eq. m^{-2} y^{-1}, suggesting that SRC willow was a slight source. The growing season for 2009 also

reflected similar values. For the growing season of 2010, SRC willow was shown to be a weak sink for N_2O with a GWP of -0.6 g CO_2 eq. m^{-2} y^{-1}. Overall, SRC willow was a slight source for GHG but that the contribution of CH_4 and N_2O are minimal to the overall budget.

Table 2.4 - A soil greenhouse gas (GHG) budget for SRC willow showing the mean GHG emissions and global warming potentials (GWP) for 2009, the growing season (GS) of 2009 and the GS of 2010. GWP were calculated for CH_4 and N_2O based on the values 25 and 298 respectively (IPCC, 2007a).

	CO_2	CH_4	N_2O	GWP: $CO_2 + CH_4 + N_2O$
Annual 2009				
GHG (g m^{-2} y^{-1})	699.01	-0.03	1.42	
GWP (g CO_2 eq. m^{-2} y^{-1})	699.01	-0.75	3.72	**702**
Growing Season 2009				
GHG (g m^{-2} GS^{-1}) ‡	915.65	-0.04	0.01	
GWP (g CO_2 eq. m^{-2} GS^{-1}) ‡	915.65	-1.09	2.02	**917**
Growing Season 2010				
GHG (g m^{-2} GS^{-1}) ‡	595.73	-0.002	-0.002	
GWP (g CO_2 eq. m^{-2} GS^{-1}) ‡	595.73	-0.06	-0.60	**595**

‡ Growing season (GS) is define here as March to September.

2.5 Discussion

During the course of this 2-year measurement campaign, monthly mean soil CO_2 fluxes ranged from 10.4 to 243.4 mg CO_2-C m^{-2} h^{-1} and there appeared to be very little difference between measurements taken IR or BR. There was a clear seasonal trend in the CO_2 flux which was largely explained by soil temperature (r^2=0.86, logged CO_2). The positive relationship between temperature and CO_2 flux has been reported in many temperate agricultural studies (Verma *et al*., 2005; Omonode *et al*., 2007; Regina & Alakukku, 2010) and is largely due to an increase in soil microbial activity and enhanced root respiration during the warmer growing season. Other studies under SRC willow report a similar but weaker relationship with temperature (Drewer *et al*., 2011, r^2=0.34; Gauder *et al*., 2011, r^2=0.55). Soil moisture content and WFPS negatively influenced soil CO_2 fluxes, with r^2= -0.58 and r^2= -0.50, respectively (Figure 2.4). This differs from the findings of Gauder *et al*., 2011, who reported a positive relationship with soil moisture although it was a relatively weak relationship (r^2= 0.11). Others studies suggest that at very low and very high soil moistures, CO_2 fluxes are reduced (Bowden *et al*., 1998), suggesting that CO_2 fluxes from under our SRC willow are low due to high soil water content but are at their optimum when soil moisture content is between 15 and 30% and WFPS was lower than 60%. At higher soil moisture contents and WFPS, CO_2 fluxes decline due to due to increasing anaerobic conditions suppressing microbial activity. However, the effects of soil moisture on CO_2 flux are often influenced by soil temperature and it is difficult to separate the effects of these parameters in the field (Bowden *et al*., 1998). Our results do suggest an interaction of these parameters, within the highest CO_2 flux at the highest temperatures and lowest soil moistures and WFPS. The positive relationship between soil C content and CO_2 flux (r^2= 0.35), suggests that substrate availability

may also play a role in CO_2 flux. Enzyme-catalysed reactions in microbes during decomposition of litter or SOM are not only affected by temperature but also by substrate availability and when substrate availability is low, for example, this may result in low soil CO_2 flux (Davidson & Janssens, 2006). The main source of readily available C in SRC willow is from the litter (Baum *et al*., 2009) and other studies investigating the SRC species suggest that annual litterfall could be between 533 and 769 g m^{-2} (Calfapietra *et al*., 2003), making this an important source of nutrients and potentially influencing in CO_2 flux. However, due to the lack of disturbance and lack of incorporation of litter into the soil, decomposition could be slow, so the contribution to the overall CO_2 flux could be low (Holland & Coleman, 1987).

Mean CH_4 fluxes were generally low with fluxes ranging from -9.7 to 32.2 µg CH_4-C m^{-2} h^{-1} over the 2-year study period and there was no significant difference between measurements taken IR or BR. In general, our study showed that SRC willow was a weak sink for CH_4 with mean annual net CH_4 uptake of -8.3 µg C m^{-2} h^{-1}. This is in agreement with other agricultural studies reporting uptake rates of -6.0 µg C m^{-2} h^{-1} (Gauder *et al*., 2011) and contrasts forested systems where the highest CH_4 oxidation rates are observed (Priemé & Christensen, 1997a; Price *et al*., 2004b). Low CH_4 oxidation has been reported in other studies looking at SRC willow, which suggest that disturbance can have undesirable effects of methanotrophic bacteria and therefore reduce the CH_4 oxidation potential of the soil (MacDonald *et al*., 1996; Priemé *et al*., 1997b; Hütsch, 2001). It is generally thought that soil disturbance events can reduce the soil CH_4 sink for decades (Smith *et al*., 2000), which may explain why the three-year management cycle of harvesting may still impact CH_4 oxidation rates at the end of the three-year rotation (Castro *et al*., 2000). Being a soil previously in arable rotation with N fertilisation (another inhibitor of CH_4 oxidation) a lag in recovery in

CH_4 oxidation rates is unsurprising. The drivers behind CH_4 flux in this study are difficult to determine due to the negligible fluxes observed. A very weak positive (r^2 = 0.1) relationship between CH_4 flux and soil moisture and WFPS was observed. Control of CH_4 flux by soil moisture has also been reported by many other studies (Bowden *et al*., 1998; Hütsch, 2001; Schaufler *et al*., 2010).

In our study, monthly mean N_2O fluxes were negligible and across the whole 2-year period ranged from -6.3 to 4.8 µg N_2O-N m^{-2} h^{-1}. There was a weak, positive relationship between N_2O emissions and soil moisture and WFPS, although like CH_4 emissions, this was not significant. WFPS was less than 60% on only 7 out of 21 months where WFPS was recorded, which may suggest that conditions were much more favourable for denitrification than nitrification (Dobbie & Smith, 2002). Low N_2O emissions have also been reported by other studies where no fertiliser was applied (Kavdir *et al*., 2008; Gauder *et al*., 2011; Drewer *et al*., 2011). The overall low N_2O fluxes are likely to also be facilitated by the nature of the crop itself, since perennial, high biomass crops have a higher N efficiency resulting in less available N in the soil and therefore lower N_2O emissions (Kavdir *et al*., 2008).

In comparison to other perennial bioenergy crops, our results would suggest that SRC willow has similar GHG (CO_2, CH_4 and N_2O) fluxes to other SRC such as *Populus* species and natural forest (Ferre *et al*., 2005; Ambus & Robertson, 1999), although our CO_2 fluxes tended to be lower than both these crops. *Miscanthus*, a perennial grass species, also had similar results to SRC willow, in terms of GHG emissions in unfertilised plots, but this is from a limited number of studies (Jørgensen *et al*., 1997; Drewer *et al*., 2011; Gauder *et al*., 2011; Chapter 3). SRC willow generally has significantly lower N_2O emissions than arable crops, largely due to relatively greater fertiliser addition to arable crops (Jørgensen *et al*., 1997; Hellebrand *et al*., 2003;

Kavdir *et al*., 2008; Drewer *et al*., 2011). Where experiments have been carried out looking at the effects of fertiliser addition on GHG emissions the results have been mixed, with some studies reporting no significant increase in N_2O emissions (Gauder *et al*., 2011) and others showing that fertiliser derived N_2O constitute as much as 32% to total measured N_2O flux (Hellebrand *et al*., 2003; Kavdir *et al*., 2008). However, SRC willow does tend to show lower N_2O fluxes with fertiliser addition than *Miscanthus* and Maize (Drewer *et al*., 2011; Gauder *et al*., 2011), which may be a considerable advantage to SRC willow if management practices change in the UK. If SRC willow does require fertiliser additions, it has been suggested that N_2O emissions could be reduced if waste water or sludge was used as an alternative to fertilisers (Dubuisson & Sintzoff, 1998).

2.6 Conclusions

The results of this study indicate that CO_2 flux was the main contributor to the overall soil GHG budget with minimal inputs from CH_4 and N_2O. The clear seasonal pattern associated with CO_2 flux and significant correlations found with temperature, soil moisture and soil C content should be useful for future modelling of these soil emissions. Overall, more research is needed to understand the longer-term dynamics of the whole system C balance to the farm gate under bioenergy crop production systems using techniques such an eddy flux covariance. Collectively, such results are required to provide useful information to ensure that the most sustainable pathways are chosen for future bioenergy expansion.

2.7 Acknowledgements

We would like to thank the land owner, Jonathan Wright, for allowing access to his land; everyone who assisted with fieldwork, with special thanks to Simon Oakley, Laura Barringer and Nicola Thompson at CEH; Helen Quirk and Richard Bardgett for allowing the use of the facilities at Lancaster University for DOC analysis; Phil Rowland and Clive Woods for the use of laboratory equipment at CEH; Julia Drewer and Ute Skiba at CEH for advice and help with initial site set-up. This work was funded under the CEH Environmental Change Integrating Fund Project no C03746.

Chapter 3

Greenhouse gas emissions from soils under a UK *Miscanthus x giganteus* plantation.

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Declaration of contribution

I declare that this chapter consists of original work undertaken by myself. The contribution of co-authors is as such. Simon Oakley and Niall McNamara assisted with fieldwork and Jon Finch provided continuous environmental data (temperature and soil moisture) from a nearby meteorological station, Niall McNamara, Jon Finch and Pete Smith provided PhD supervision and comments on this manuscript.

3. 1 Abstract

Miscanthus x giganteus is one of the most promising bioenergy crops in the UK and has the potential to reduce greenhouse gas (GHG) emissions from energy producing systems as an alternative to fossil fuels. GHG emissions (CO_2, CH_4 and N_2O) from soils are often sparse and limit our ability to accurately assess GHG mitigation potential of these crops. The aim of this study was to quantify CO_2, CH_4 and N_2O emissions from soils, and determine the main environmental and soil chemical drivers behind these fluxes from a *Miscanthus* plantation in Lincolnshire, UK. Measurements were taken on a monthly basis from October 2008 to November 2011. Mean monthly CO_2 emissions ranged from 8 to 633 mg CO_2-C m^{-2} h^{-1} and showed a clear seasonal pattern with significant correlations found with soil temperature (r=0.81), and soil moisture (1-15 cm depth, r=-0.77). There was limited evidence that soil moisture may have limited CO_2 fluxes at times of high temperatures. Fluxes of CH_4 and N_2O were minimal with fluxes ranging from -13.7 to 16.9 µg CH_4-C m^{-2} h^{-1} and -7.4 and 11.8 µg N_2O-N m^{-2} h^{-1} respectively. CH_4 fluxes showed a significant correlation with soil moisture (0-6 cm, r=0.54) and there was some evidence of N_2O fluxes being influenced by soil moisture (0-15 cm), but more evidence is needed to confirm this. Overall, the soil GHG budget showed that CO_2 was the main flux from soil, with CH_4 and N_2O only making a minor contribution to the overall budget.

3.2 Introduction

Miscanthus x giganteus (hereafter *Miscanthus*) is one of the most promising bioenergy crops in the UK, covering approximately 12,700 hectares (ha) (DEFRA, 2009). Although native to tropic and sub-tropic regions of Asia, *Miscanthus* has shown considerable biomass potential even under temperate conditions (Naidu *et al*., 2003) and can achieve yields of between 10-20 t ha^{-1} (Clifton-Brown *et al*., 2007; Gauder *et al*., 2012). Previous research has focussed on propagation, establishment, and management practices to improve yields (Lewandowski *et al*., 2000) but concerns around energy security together with a warming climate caused by the use of fossil fuels, have increased interest in *Miscanthus* over the past decade to provide a low carbon (C) renewable energy source. *Miscanthus* is currently used in electricity and heat production to reduce greenhouse gas (GHG) emissions relative to fossil fuels but recent advances in cellulosic ethanol technology, may also see this crop converted into transport fuel (Heaton *et al*., 2008). This could lead to an expansion in growing *Miscanthus* internationally but the full impacts of this are not well quantified in terms of the soil GHG fluxes and soil carbon sequestration. There is a need to fully understand the GHG mitigation potential of this crop.

Agricultural soils in Europe make up more than half the GHG emissions produced from the agricultural sector (UNFCCC, 2011), with the most important GHG being carbon dioxide (CO_2), methane (CH_4) and (N_2O) (Smith *et al*., 2007). Soil CO_2 fluxes are a balance between C fixed through photosynthesis and the return of CO_2 to the atmosphere through plant respiration (leaves, stems and roots) and microbial respiration during the decomposition of organic matter (Trumbore, 2006). The status of soil to be an overall sink or source therefore depends on the balance of photosynthesis and respiration. Net CH_4 fluxes result from the simultaneous processes

of methanogenesis and methanotrphy under anaerobic and aerobic conditions respectively (Smith *et al*., 2003). N_2O production in soils is linked to two contrasting processes of nitrification and denitrification, in the presence of ammonium (NH_4^+) and nitrate (NO_3^-) under aerobic and anaerobic conditions respectively (Smith *et al*., 2003). Both CH_4 and N_2O fluxes are lower than that of CO_2, but the global warming potential (GWP) of these gases is 25 and 298 times more powerful than CO_2, making them highly important to the overall GHG balance of soils (IPCC, 2007a).

Quantifying GHG fluxes from agricultural landscapes is difficult since the underlying processes are influenced by so many different factors including temperature, moisture, soil type, crop planted, and management practices used (Mosier *et al*., 1991; Smith & Conen, 2004). Ideally, GHG measurements should be made from a variety of sites, which encompass different environmental conditions and management practices, to improve our understanding of the driving forces behind these fluxes and in turn our estimates of these fluxes. For *Miscanthus*, field-based measurements of fluxes are sparse and many of the published studies regarding GHG fluxes from this crop are from life cycle analyses (LCA), which use default values from the IPCC (Hillier *et al*., 2009; Whitaker *et al*., 2010; Brandão *et al*., 2011), or are from modelled fluxes from soils (Dondini *et al*., 2009). From the limited field based data available, the emissions of CH_4 and N_2O from *Miscanthus* planted soils are negligible (Guader *et al*., 2011; Drewer *et al*., 2011). The latter is largely due to little or no additions of fertiliser for the management of this crop, with *Miscanthus* having a number of physiological attributes that allow it to recycle nutrients at the end of the growing season (Beale & Long, 1997; Lewandowski *et al*., 2003; Heaton *et al*., 2004). Furthermore, it is suggested that *Miscanthus* may harbour free living bacteria capable of N fixation in the rhizome (Eckert *et al*., 2001; Miyamoto *et al*., 2004; Davis *et al*., 2010).

3. GHG emissions from *Miscanthus*

While model predictions show that *Miscanthus* has advantages over other bioenergy crops, it is clear that more field-based research is required to validate and parameterise these model outputs. The aim of this study was to firstly quantify soil fluxes of CO_2, CH_4 and N_2O from *Miscanthus* over two growing seasons and secondly to investigate the main environmental and soil chemical drivers behind these fluxes. A further aim would be to develop relationships that may be useful in modelling these fluxes and provide information that could be useful for life cycle analysis and soil C models.

3.3 Materials and Methods

3.3.1 Study Site and Experimental Design

The study was conducted within a *Miscanthus* bioenergy plantation (11 ha) located in Lincolnshire, UK. The underlying soil type was a clay loam with approximately 25, 29 and 49% clay, sand and silt, respectively. The soil had a mean C and N content of 1.5% and 0.3% respectively, and pH that ranged from 6.8 to 7.3. Water-filled pore space (WFPS) ranged from 82.4 in 2009 and 71.4 in 2010, and there are further soil properties shown Table 3.2. The site had a 30-year annual mean precipitation of 605 mm and temperature of 9.9 °C (Table 3.3).

The *Miscanthus* was established in 2006 at a density of 10,000 rhizomes ha^{-1}. The crop has been harvested annually, with yields of 5, 10 and 6 t ha^{-1} for 2009, 2010 and 2011 respectively. The only addition of fertiliser was in April 2010, when a PK fertiliser was applied at a rate of 125 kg ha^{-1}. The land management prior to land conversion to *Miscanthus* was a crop rotation of wheat and oil seed rape, with three years of wheat directly before conversion.

Five randomly dispersed sampling blocks were established, with a minimum distance between plots of 50.6 m and a maximum of 218.7 m from which to take our measurements. These measurements included soil-atmosphere trace gas fluxes (CO_2, CH_4 and N_2O), soil solution chemistry (DOC, NH_4^+, NO_3^-), total C and N stock and the associated climatic measures (soil and air temperature and soil moisture). Samples were taken on a monthly basis over a period of 25 months from October 2008 to November 2010. Please see previous chapter (section 2.2.2-2.3.4) for full details on the method used for soil-atmosphere GHG fluxes and soil and environmental measurements.

3.3.2 Statistical Analysis and Soil GHG Budget

The Statistical package R (version 2.14.0) was used for all analysis. Monthly mean values for all fluxes, soil and environmental parameters were tested for normality and were transformed where appropriate. In the case of N_2O and CH_4 fluxes a constant of 10 and 15 was added respectively before normality testing as these were equivalent to the lowest measured flux of each gas. Pearson product moment correlation was used to relate gas flux data to soil and meteorological data when normally distributed. Theta probe measurements could not be obtained from the field in July 2009, July 2010 and August 2010 as the probe could not be inserted into the clay soil due to drought conditions in the field.

Linear mixed effects models were used to relate meteorological and soil data to CO_2 flux, with 'date' used as a random effect to account for repeated measurement over time. This was done by starting with the most relevant soil and environmental parameters as determined from the correlation analysis and then removed sequentially, starting with the least significant, until only significant parameters remained. Four combinations of temperature and soil moisture were used with other soil and meteorological parameters; **a)** soil temperature and Theta moisture, **b)** soil temperature and gravimetric moisture, **c)** air temperature and Theta moisture and, **d)** air temperature and gravimetric moisture. The best model fit was determined by the AIC and model graphical outputs. The significance of all results was accepted when $p \leq 0.05$.

The final model produced from the linear mixed model (Table 3.3) was used to infer hourly CO_2 flux from the continuous air temperature and soil moisture measurements collected from the nearby meteorological station. There was good agreement between continuous air temperature and measured air temperature (r^2=0.91) and between

continuous soil moisture measurements (0-20 cm) and measure gravimetric moisture (r^2=0.87). The modelled CO_2 flux values were used in the annual and growing season soil GHG budget for *Miscanthus*. Due to the minimal fluxes measured for CH_4 and N_2O, the monthly mean values were averaged and then extrapolated to calculated values for the soil GHG budget. CH_4 and N_2O fluxes were converted to CO_2 equivalents (CO_2 eq.) based on their global warming potential of 25 and 298 for CH_4 and N_2O respectively (100 year time scale, IPCC, 2007a).

3.4 Results

3.4.1 Field Climatic Measures

Precipitation varied from 0 to 34 mm up to 7 days before sampling (Figure 3.1b), with the highest values recorded in October 2010. The precipitation for 2010 and 2009 were close to the 30-year average precipitation of 605 mm, with 2009 receiving slightly higher precipitation and 2010 receiving slightly lower precipitation (Table 3.1). The precipitation for the growing season followed a similar pattern.

Table 3.1 - Summary of climate data, including precipitation, air temperature and 30-year average for *Miscanthus x giganteus* Lincolnshire, UK. Annual values for 2010 were not included as it was an incomplete year of measurements.

Year	Max. Air Temp. (°C)	Min. Air Temp. (°C)	Mean Air Temp. (°C)	Precipitation (mm)
Annual				
2009	30.0	3.5	12.8	679
2010	-	-	-	545
30 year average†	25.8	-3.7	9.9	605
Growing Season‡				
2009	30.0	9.0	16.9	464
2010	28.5	11.7	18.5	357
30 year average†	25.8	14.4	13.2	387

†30-year average was from 1980 to 2010 taken from a nearby meteorological station
‡Growing season taken from April to October.

Air temperatures ranged from 0.8 to 30°C, with minimum temperatures seen in January of both years and maximum temperatures in July of both years (Figure 3.1a). Soil temperatures followed a similar pattern, however minimum values were in February of both years and ranged from 0.5 to 23.5°C. The mean annual temperatures and the growing season temperatures for 2009 and 2010 were both higher than the 30-

year average (Table 3.1). Maximum air temperatures for 2009 and 2010 were close to that of the 30-year average of 25.8°C, however the growing season minimum temperatures were lower than the 30-year average of 14.4°C (Table 3.1).

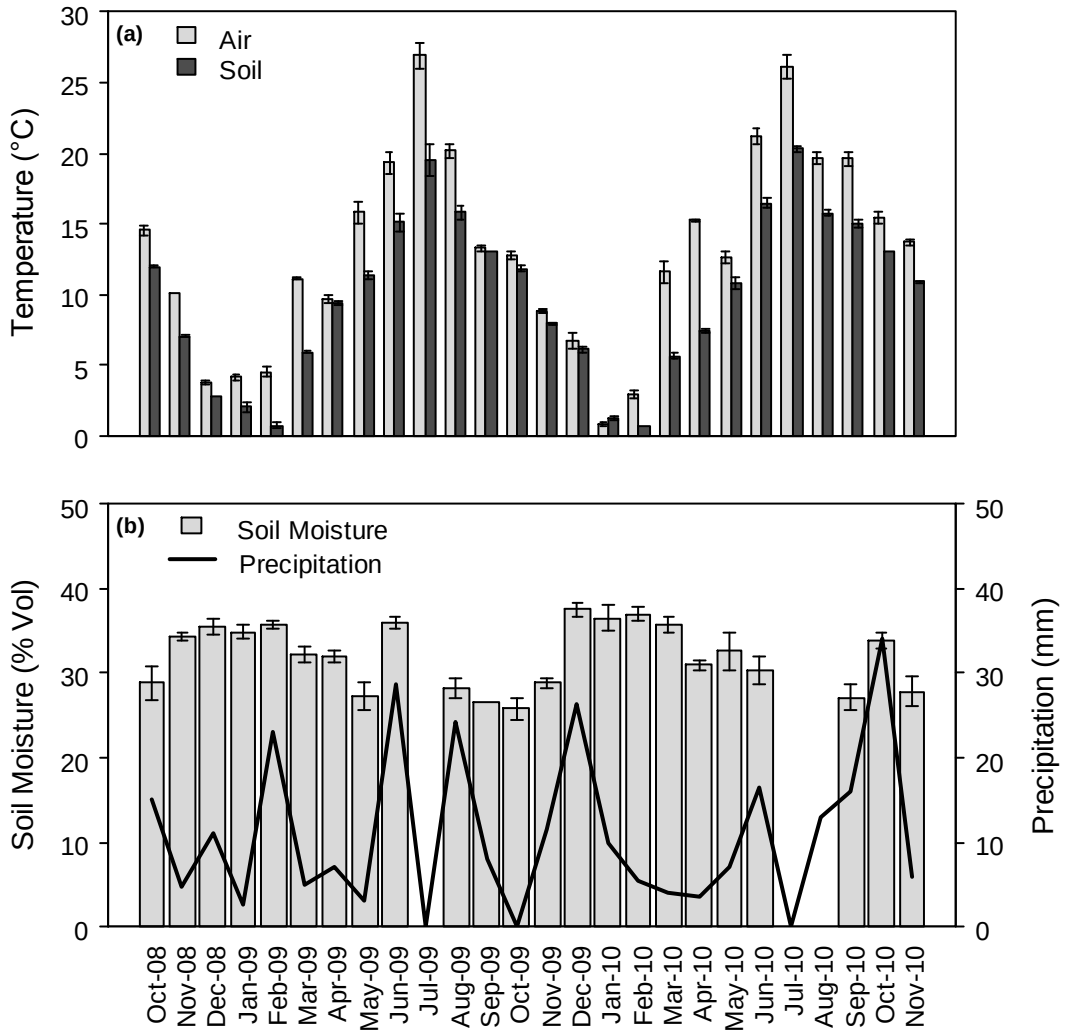

Figure 3.1 - Mean Monthly (n=5) environmental parameters from October 2008 to November 2011. **a)** Air Temperature (light grey bars) and soil temperature (dark grey bars), **b)** Soil moisture (Theta- 0-6 cm) and total precipitation 7 days before sampling. Soil moisture is missing from Jul-09, Jul-10 and Aug-10 as soil was too dry for insertion of Theta probe. Error bars represent standard error values.

3.4.2 Soil Physical and Chemical Properties

Soil cores collected on a monthly basis were used to determine a number of physical characteristics that are shown in Table 3.2. Soil BD, total C and N remained constant over the study. Theta moisture (0-6 cm depth) ranged from 26.6% to 37.6% and was generally above 30% from November to June and fell below 30% during periods of higher temperature over the summer and autumn (Figure 3.1b). Monthly mean values of WFPS varied from 44.9 to 108.2%, and was higher in 2009 than 2010 largely due to peak WFPS in June 2009 (Table 3.2).

Table 3.2 - Summary of soil properties (0-15 cm, depth) from *Miscanthus x giganteus*, Lincolnshire, UK. Values are an overall mean of mean monthly values (n=5) with standard errors values.

Parameter	2009	2010
Bulk Density (g cm^{-3})	1.49 ± 0.02	1.40 ± 0.02
Total C (%)	1.52 ± 0.02	1.55 ± 0.06
Total N (%)	0.25 ± 0.002	0.26 ± 0.06
C:N Ratio	6.01	6.07

Soil DOC concentrations ranged from 21.3 to 141.2 µg g^{-1} dw soil with a mean of 81.67 µg g^{-1} dw soil (Figure 3.2a). No seasonal pattern was observed and no significant correlations were found with any soil or environmental parameters. Mean soil NH_4^+-N concentrations ranged from 0.6 to 2.5 mg kg^{-1} and remained relatively constant throughout the study period, with only one significant correlation found with gravimetric moisture ($r=-0.58$, $p<0.05$) (Figure 3.2b). Mean soil NO_3^--N concentrations ranged from 0.8 to 4.3 mg kg^{-1}, peaked in both years in July, and generally remained higher than 3.0 mg kg^{-1} until October 2009 and September 2010 (Figure 3.2c). NO_3^--N concentrations were found to be significantly correlated with

Theta moisture (0-6 cm depth, r = -0.56, $p<0.05$) and gravimetric moisture (0-15 cm depth, r = -0.69, $p<0.01$).

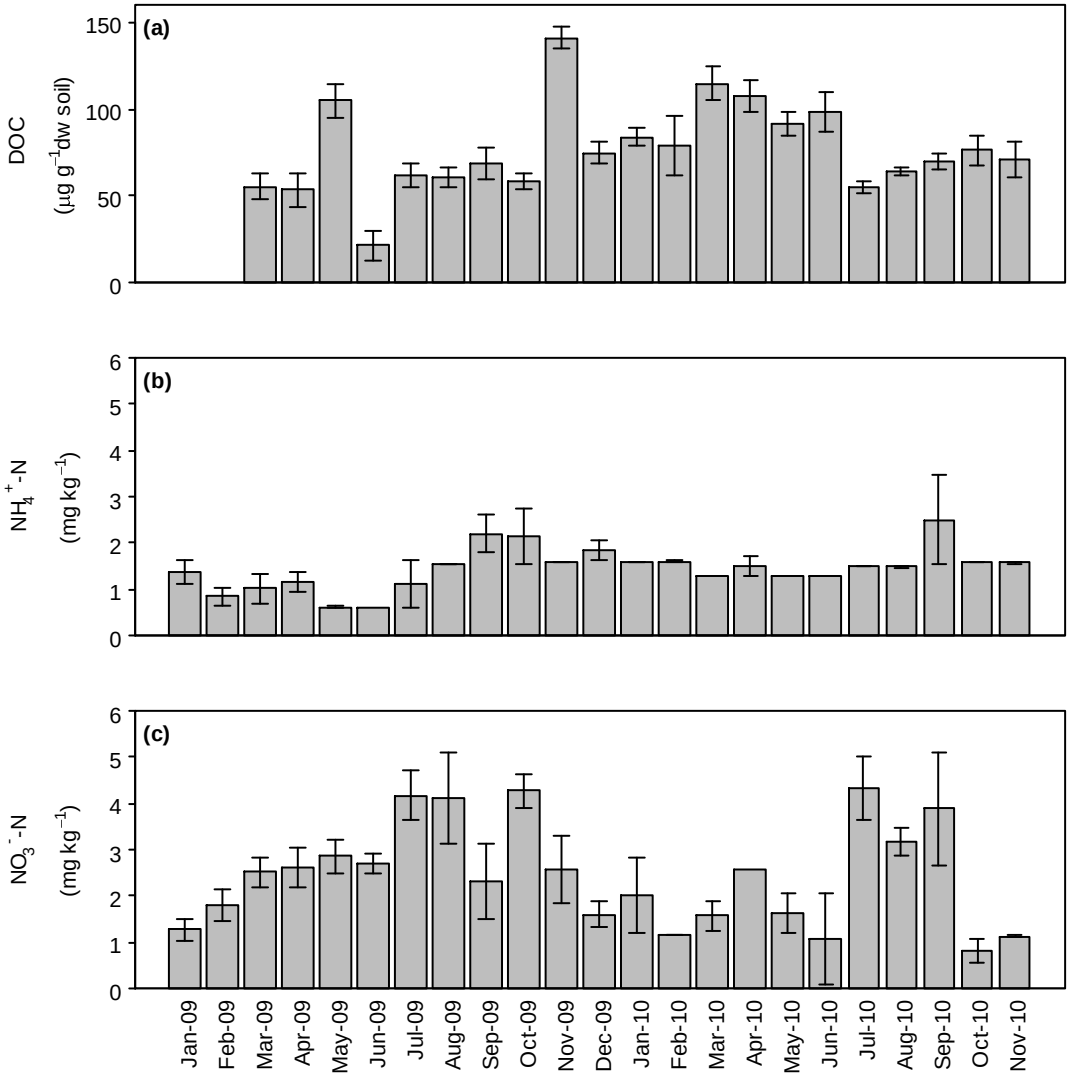

Figure 3.2 - Mean monthly (n=5) soil chemical properties, **a)** soil extractable DOC, **b)** soil extracted NH_4^+-N and **c)** soil extracted NO_3^--N. Error bars represent standard error values.

3.4.3 Soil GHG Fluxes

The measured CO_2 fluxes showed a strong seasonal pattern, which largely followed changes in temperature (Figure 3.3). Lower fluxes were observed in the winter and were typically between 10 and 20 mg CO_2-C m^{-2} h^{-1}. Higher fluxes were measured in the summer with a peak respiration rate of 633.1 mg CO_2-C m^{-2} h^{-1} in July 2009 (Figure 3.3). There appeared to be no change in CO_2 emissions after fertiliser (PK) addition in April 2010. There were several strong correlations found with environmental parameters, which are shown in Table 3.3.

Table 3.3 - The meteorological and soil properties that showed significant correlations with CO_2, CH_4 and N_2O flux. Values are the Pearson production-moment correlation coefficient (r) and the associated significance (p value). N_2O fluxes show correlations with and without June 2010 peak. Levels of significance are $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***) for F values at 1, 16 *df* for CO_2 and CH_4 and 1, 18 *df* for N_2O.

Parameter	Air Temp	Soil Temp	Theta Moisture‡	Gravimetric Moisture†
CO_2 Flux				
r	0.75	0.81	-0.70	-0.77
F value	20.06 (***)	30.85 (***)	15.56 (**)	22.95 (***)
CH_4 Flux				
r	-	-	0.54	-
F value	-	-	0.02 (*)	-
N_2O Flux				
r	-	-	-	0.28 (0.44) §
F value	-	-	-	1.63 (ns) (4.30 (*)) §

‡ 0-6 cm depth and † 0-15 cm depth

§ Figures in parentheses show values without high N_2O peak (June 2010) included in analysis

ns = not significant

The strongest correlation was with soil temperature (r=0.80), followed by gravimetric moisture, air temperature, and Theta moisture. The strongest of these relationships are shown in Figure 3.4, with soil temperature explaining 70% of the variation and soil moisture and WFPS explaining about 62% of the variation. No significant correlations were found with NO_3^- ($p<0.05$), NH_4^+ ($p<0.05$) or DOC ($p=0.08$). Linear mixed effects models showed that air temperature and gravimetric moisture best described soil CO_2 flux (Table 3.4). Other model combinations also indicated that temperature and soil moisture were the best parameters for describing CO_2 flux, but the model fit was less good.

Table 3.4 - Environmental and soil properties were used to test which parameters were most important in describing CO_2 flux. Linear mixed models were used by sequentially removing parameters until only significant ones were left, with the best model fit is shown here. All fixed parameters were accepted as significant when $p < 0.05$.

Variable	DF	F-value	p-value
CO_2 Flux† = (Air Temp * 0.0349226) + (Grav Moisture * -0.0601032) + 2.5106824			
Air Temperature	19	55.09	<0.001
Gravimetric Moisture	19	8.21	<0.01

† Log transformed

Mean monthly CH_4 fluxes were varied between -13.7 and 16.9 μg CH_4-C m^{-2} hr^{-1} and generally followed changes in soil moisture and precipitation, with CH_4 oxidation generally occurring when soil moisture was below 30%. This is supported by a significant correlation being found with Theta moisture (r=0.54) (Table 3.3), however, linear mixed effects models showed no significant environmental or soil properties to influence CH_4 fluxes.

The majority of N_2O fluxes ranged between -7.4 and 11.79 µg N_2O-N m^{-2} hr^{-1} but a high flux was observed in June 2010 of 47.9 µg N_2O-N m^{-2} hr^{-1}. This may be linked to soil moisture since a weak correlation was found with gravimetric soil moisture (r=0.28) (Table 3.3), but since this was the only large flux seen it is difficult to determine the exact reasons for this flux. Linear mixed effects models indicated that gravimetric moisture may influence N_2O emissions but the model fit was poor.

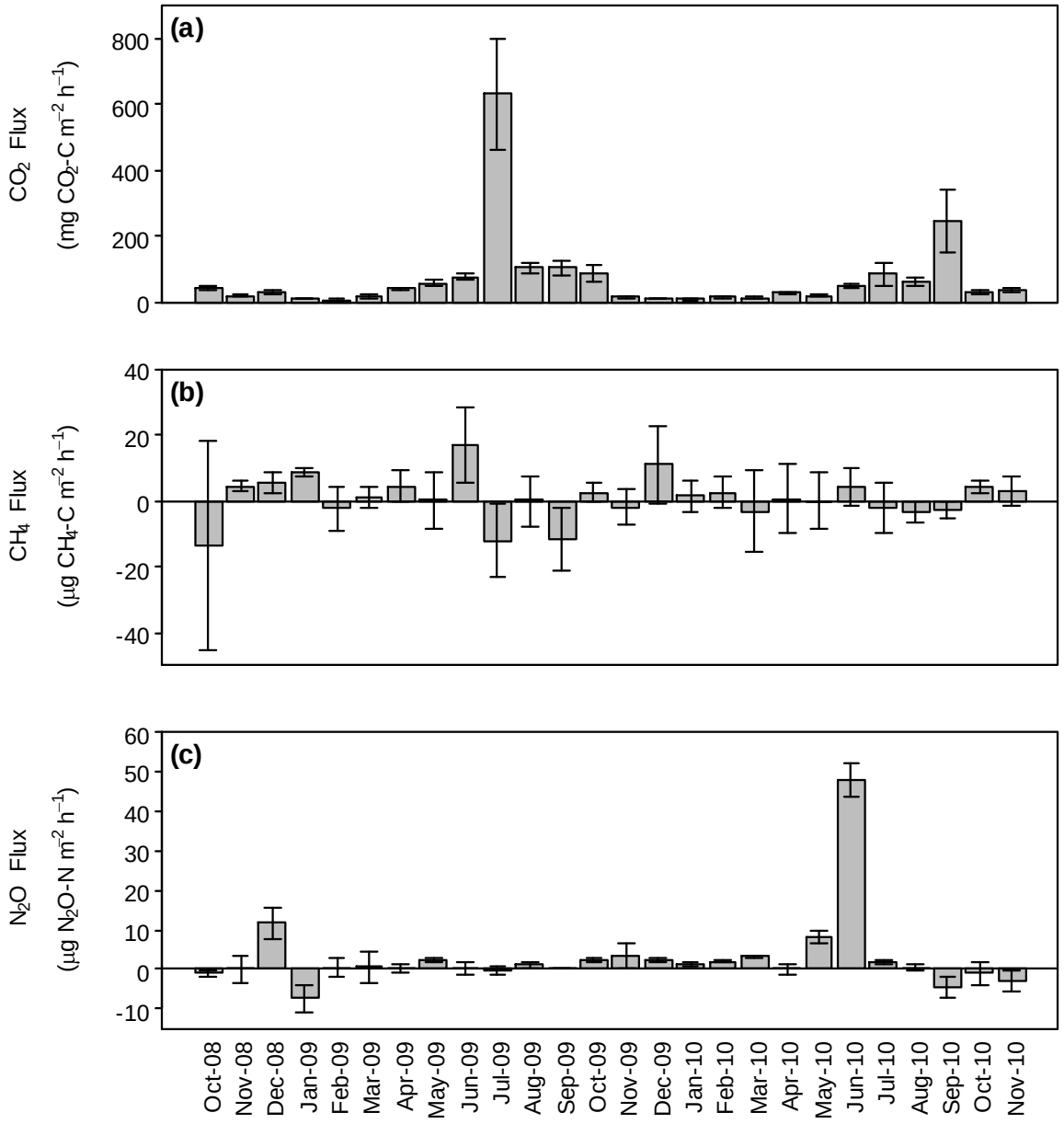

Figure 3.3 - Mean monthly (n=5) GHG fluxes, **a)** soil respiration, **b)** CH_4 flux, **c)** N_2O flux. Error bars represent standard error values.

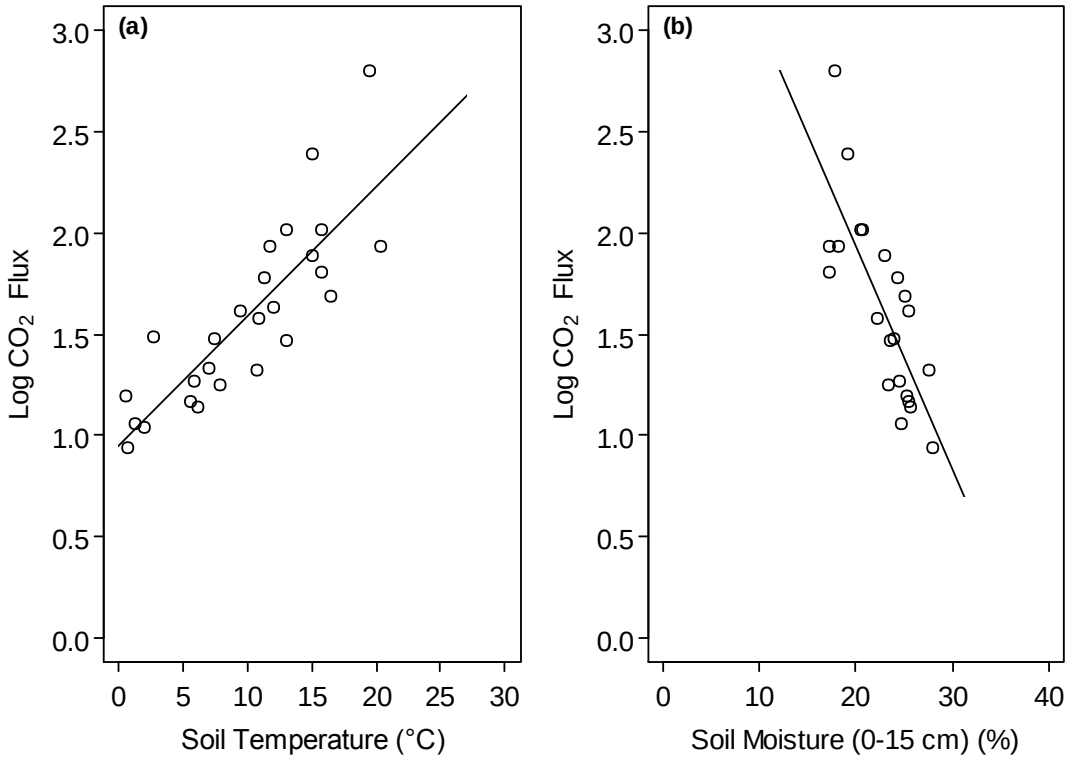

Figure 3.4 – Correlation between logged CO_2 flux and **a)** soil temperature and **b)** gravimetric moisture (0-15 cm depth). Values are mean monthly soil respiration rates, soil temperature and gravimetric moisture.

3.4.4 Soil GHG Budget

The soil GHG budget was calculated on a CO_2 equivalent basis and an annual budget and 'growing season' budget for 2009 and 2010 is shown in Table 3.5. CO_2 fluxes ranged from 860 to 1000 g CO_2 eq. m^{-2} y^{-1} and were clearly the main contributor to the overall GHG budget. *Miscanthus* soils were shown to be a slight source of CH_4 since all values were positive in all periods shown (Table 3.5), but fluxes were minimal and only ranged from 0.001 to 0.02 g CH_4 m^{-2} y^{-1}. Consequently, the GWP were also minimal with the highest value of 0.56 g CO_2 eq. m^{-2} y^{-1} from 2009. *Miscanthus* soils were also a slight source for N_2O but this varied dramatically between years. The annual GWP value for 2009 was 1.28 g CO_2 eq. m^{-2} y^{-1}, which increased to 2.35 g CO_2 eq. m^{-2} y^{-1} over the growing season. However, due to the high flux measured in June 2010, the GWP for the growing season in 2010 was 19.76 g CO_2 eq. m^{-2} y^{-1} (Table 3.5). Overall, *Miscanthus* soils were a slight source for GHGs, with CH_4 contributing little to the overall budget. N_2O fluxes appeared to be much more changeable and so contributed only 2.5% to the overall in 2009 (growing season) but 17.5% in 2010 (growing season).

Table 3.5 - Soil greenhouse gas (GHG) budget for 2009 and 2010 for *Miscanthus*. CO_2 values are based on modelled hourly fluxes using continuous air temperature and soil moisture (0-20 cm depth). CH_4 and N_2O values were extrapolated from monthly mean data that had been averaged over the year or over the growing season. CH_4 and N_2O fluxes were converted into CO_2 equivalents (CO_2 eq.) using global warming potentials (GWP) of 25 and 298 for CH_4 and N_2O respectively (IPCC, 2007a).

	CO_2	CH_4	N_2O	GWP: $CO_2 + CH_4 + N_2O$
Annual 2009				
GHG (g m^{-2} y^{-1})	1001.7	0.02	0.004	
GWP (g CO_2 eq m^{-2} y^{-1})	1001.7	0.56	1.28	**1003.5**
Growing Season 2009				
GHG (g m^{-2} GS^{-1})‡	863.7	0.02	0.01	
GWP (g CO_2 eq m^{-2} GS^{-1}) ‡	863.7	0.44	2.35	**866.5**
Annual 2010				
GHG (g m^{-2} y^{-1})	990.1	-	-	
GWP (g CO_2 eq m^{-2} y^{-1})	990.1	-	-	
Growing Season 2010				
GHG (g m^{-2} GS^{-1}) ‡	929.7	0.001	0.07	
GWP (g CO_2 eq m^{-2} GS^{-1}) ‡	929.7	0.02	19.76	**949.5**

‡GS is Growing Season values from April to October

3.5 Discussion

During the course of this two-year study soil respiration ranged from 8.8 to 633.1 mg CO_2-C m^{-2} hr^{-1} but the majority of fluxes were below 250 mg CO_2-C m^{-2} hr^{-1}. These results were comparable with Gauder *et al*., (2011) and Drewer *et al*., (2011) who reported fluxes in the range of 0.3 to 217 mg CO_2-C m^{-2} hr^{-1} and 0 to 0.47 g m^{-2} h^{-1} (equivalent to 475.2 mg CO_2-C m^{-2} hr^{-1}) respectively from under *Miscanthus* crops. A number of studies have also been carried out in natural grasslands of *Miscanthus sinensis* in Japan, and broadly agree with our results (Wang *et al*., 2005; Toma *et al*., 2010b). Soil respiration reported from Toma *et al*., (2010b) was generally lower than those in this study (35.8 to 95.3 mg CO_2-C m^{-2} hr^{-1}) but the maximum temperature recorded was 15°C and when this is taken in to consideration, the results correspond well with our fluxes from 15°C and below (Figure 3.4). In comparison with other bioenergy crops, our results are similar to CO_2 fluxes from switchgrass (*Panicum virgatum*), which is a perennial grass primarily grown for bioenergy in the USA (Frank *et al*., 2004; Lee *et al*., 2007). Lee *et al*., (2007) reported fluxes of between 21 and 292 mg CO_2-C m^{-2} h^{-1} and Frank *et al*., (2004) CO_2 fluxes ranged from 83 to 833 mg CO_2-C m^{-2} h^{-1}, with higher fluxes in the summer months than those reported in this study. Studies investigating GHG emissions from maize, a C_4-crop also used for bioenergy, suggest that CO_2 fluxes are similar, if a little lower, to the ones found in this study, in the region of 40 to 250 mg CO_2-C m^{-2} h^{-1} (Rochette *et al*., 1999; Ding *et al*., 2007).

There was a strong seasonal pattern to soil respiration, which generally matched that of temperature and is reflected in the significant correlations found with both air and soil temperature in this study. The influence of temperature on soil respiration has been well documented across a range of different ecosystems (Raich & Schlesinger,

1992; Lloyd & Taylor, 1994) and was also found in studies investigating *Miscanthus* (Yazaki *et al*., 2004; Toma *et al*., 2010a; Drewer *et al*., 2011; Guarder *et al*., 2011).

There was a strong negative correlation between soil respiration and soil moisture (both 0-6 cm and 0-15 cm depth r^2 =0.62), but this was not reported by Toma *et al*., (2010b) or Drewer *et al*., (2011). However, Guarder *et al*., (2011) found a positive correlation with soil moisture, which differs from results in this study. Their correlation was a combination of all their data from three different crops and when looking at their WFPS data for *Miscanthus*, it would suggest higher CO_2 fluxes at low WFPS (and *vice versa*), which is more in line with our results. There is likely to have been a interaction between soil temperature and soil moisture, since low soil moisture content corresponded with periods of high temperature, and could have easily limited soil respiration through soil moisture being too low for microbial activity (Bowden *et al*., 1998). This is supported by Yazaki *et al*., (2004) who found much higher soil respiration rates at higher temperatures (up to 20°C), (up to 1772 mg to CO_2-C m^{-2} h^{-1}), but soil moisture contents to be around above 25% even in the summer, due to a high annual precipitation of 1288 mm, more than double than reported in this study.

Our study showed that CH_4 fluxes were minimal over the course of the two-year study ranged from -13.7 and 16.9 µg C m^{-2} h^{-1} agreeing well with the ranges reported by other *Miscanthus* studies (Toma *et al*., 2010a; Drewer *et al*., 2011; Gaurder *et al*., 2011). However, Gauder *et al*., (2011) and Toma *et al*., (2010a) both found *Miscanthus* to be an overall weak sink for CH_4, where as this study showed it to be a weak net source. Overall, the fluxes were minimal and are in support of other findings that suggest that agriculturally managed soils are not a major sink for CH_4 (Hütsch, 2001; Smith & Conen, 2004). Although *Miscanthus* is not intensively managed, it is harvested every year, which could negatively affect the activity of methanotrophic

bacteria, therefore reducing oxidation rates (Hütsch, 2001; Gauder *et al*., 2011). The soil was previously under arable crop management, including tillage and additions of fertiliser, also known to inhibit oxidising bacteria (Mosier *et al*., 1991), which would also limit the potential of these soils to be a sink for CH_4. There is also evidence that harvest residues with a narrow C:N ratio can inhibit CH_4 oxidation through enhanced N mineralisation (Hütsch, 2001). *Miscanthus* produces large amounts of litter over the senescent period, between 2 to 7 t ha-^{1} (Hansen *et al*., 2004; Amougou *et al*., 2011; Chapter 4), and tends to show narrow C:N ratios over this time (C:N 36-44, Beuch *et al*., 2000), suggesting that *Miscanthus* litter may limit CH_4 oxidation but more evidence is needed. The main driver behind CH_4 flux was soil moisture (r=-0.54), with CH_4 oxidation generally occurring at soil moisture contents of 30% and below. This was also reported by Toma *et al*., (2010a) and Drewer *et al*., (2011), which indicates that high soil moisture contents caused anaerobic conditions and promoted methanogensis (Schimel & Gulledge, 1998).

The majority of N_2O emissions reported here were between -7.4 and 11.8 µg N_2O-N m^{-2} hr^{-1} but June 2010 showed a peak of 47.9 µg N_2O-N m^{-2} hr^{-1}. Other studies have also shown minimal N_2O emissions in *Miscanthus* and in a similar magnitude to the rates found in this study (Jørgensen *et al*., 1997; Toma *et al*., 2010a; Gauder *et al*., 2011). The low emissions are not a surprise since *Miscanthus* was not N fertilised, which is known to increase N_2O emissions (Smith & Conen, 2004; Guarder *et al*., 2011). The higher emissions found in June 2010 may be linked to slightly higher periods of rainfall and increases in soil moisture, causing anaerobic conditions that may have induced denitrification (Dobbie & Smith, 2002). Toma *et al*., (2010b) also found higher rates of N_2O emissions during the summer months and attributed these to high periods of precipitation. This has also been reported from agricultural studies

using maize, with precipitation events causing high N_2O emissions (Zhang *et al*., 2012). As mentioned above, *Miscanthus* produces large amounts of litter, and there is some evidence that the way in which the litter is distributed (e.g. uniform of layered) across the soil surface could affect N_2O production and may contribute to the low fluxes seen in *Miscanthus* (Ambus *et al*., 2001; Loecke & Robertson, 2009). Results are mixed, with either possible in *Miscanthus*. Ambus *et al*., (2001) and Magid *et al*., (2006) suggest that N_2O fluxes were less when litter (agricultural residue and maize, respectively) was layered, and attributed this to N limitation and physical constraints of NO_3^- diffusion from the soil. Breland, (1994), however, suggested that uniform litter reduced denitrification due to physical protection from microbes.

3.6 Conclusion

The results show that CO_2 is the main contributor to the overall soil GHG budget with CH_4 and N_2O contributing little to the overall budget. When results are compared to other first generation bioenergy crops such as maize, where high N_2O emissions are observed (Guarder *et al*., 2011; Cui *et al*., 2012) the potential GHG mitigation potential of *Miscanthus* appears greater. Overall, second generation crops (*Miscanthus* and SRC willow – Chapter 2) appear to have a more sustainable pathway for energy generation due to lower input requirements and being perennial in nature.

3.7 Acknowledgements

We would like to thank the land owner, Jonathan Wright, for allowing access to his land; everyone who assisted with fieldwork, with special thanks to Sean Case, Laura Barringer and Nicola Thompson at CEH; Helen Quirk and Richard Bardgett for allowing the use of the facilities at Lancaster University for DOC analysis; Phil Rowland and Clive Woods for the use of laboratory equipment at CEH; Julia Drewer and Ute Skiba at CEH for advice and help with initial site set-up. This work was funded under the CEH Environmental Change Integrating Fund Project no C03746.

Chapter 4

Litter fall and litter decomposition in the bioenergy crops *Miscanthus x giganteus* and short rotation coppice (SRC) willow.

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Declaration of contribution

I declare that this chapter consists of original work undertaken by myself. The contribution of co-authors is as such. Niall McNamara assisted with fieldwork and Jon Finch provided continuous environmental data (temperature and soil moisture) from a nearby meteorological station, Clive Woods provided training and resources for the litter analyses. Niall McNamara, Jon Finch and Pete Smith provided PhD supervision and comments on this manuscript.

4.1 Abstract

We investigated litterfall and litter decomposition in two bioenergy crops *Miscanthus x giganteus* (*Miscanthus*) and short rotation coppice (SRC) willow. *Miscanthus* and SRC willow are both used as bioenergy crops to provide a low C alternative to fossil fuels, and the high litterfall from both these crops could potentially contribute to soil carbon (C) sequestration. The aim of this study was to investigate the C contribution to soil from litter, determine litter decomposition rates using the litter-bag technique, and the main drivers of decomposition using a reciprocal swap experiment in *Miscanthus* and SRC willow. *Miscanthus* litterfall period lasted from October to February-March and represented an annual input of 1.1 t C ha^{-1}. SRC willow litterfall period was more variable but in general lasted from July to January-February which also represented an annual input of 1.1 t C ha^{-1}. Both *Miscanthus* and SRC willow showed about 40% mass loss over a year, with litter decomposition mainly driven by litter quality, especially litter C content, owing to the high cellulose and lignin content of the litter. The C:N ratio was an important determinant in litter decomposition for both crops, more so for *Miscanthus* since the high C:N ratio (81) reducing initial decomposition rates due to N limitations for decomposer organisms. Overall, the results show the importance of litter in C additions to the soil and a large potential for C sequestration.

4.2 Introduction

Litterfall and subsequent decomposition are important processes within the plant-soil system and are essential in nutrient cycling in soils (Aerts, 1997). Litter decomposition plays a significant role in the global carbon (C) budget (Coâteaux *et al*., 1995) through the release of C into the atmosphere as a result of microbial breakdown of material (Raich & Schlesinger, 1992) and through C sequestration in soils through the accumulation of organic residues (Lal, 2008; Berg & McClaugherty, 2008). While litterfall and decomposition have been extensively studied in forests (Melillo *et al*., 1982; McClaugherty & Melillo 1985; Berg, 2000; Ayres *et al*., 2009), peatlands (Bartsch & Moore, 1985; Moore, *et al*., 2007) and agricultural systems (Wardle *et al*., 1999; Kochsiek *et al*., 2009), there is little emerging information in relation to non-food crops such as *Miscanthus x giganteus* (*Miscanthus* hereafter) and short rotation coppice (SRC) willow. In the UK, it is suggested that 350,000 ha (hectares) of these crops could be planted by 2020 to meet growing renewable energy demands (DEFRA, 2007).

Miscanthus and SRC willow are the two main crops grown for bioenergy in the UK, covering approximately 19,000 ha combined (Don *et al*., 2011). *Miscanthus* is a C_4 perennial, rhizomatous grass species native to subtropical regions of Asia and Africa (Lewandowski *et al*., 2000) and SRC willow is a woody species native to the UK (Karp *et al*., 2011; Rowe *et al*., 2009). For these perennial bioenergy crops, litterfall and litter decomposition are the major source of nutrients (C and nitrogen (N)) for plant growth (Zhang *et al*., 2008), especially as these systems are not usually fertilised. The litter layer also can provide a protective surface layer, which can buffer the soil from microclimatic changes in temperature, soil moisture and compaction (Sayer, 2006). Any removal of these residues to increase biomass for energy

production could therefore have detrimental effects on soil quality and crop productivity, leading to depletion of soil organic matter (SOM) and lower yields (lal, 2007; Sayer, 2006). Furthermore, both *Miscanthus* and SRC willow produce large amounts of litter, between 1 and 5 t ha^{-1} (Beuch *et al*., 2000; Baum *et al*., 2009), which tends to accumulate on the soil surface due to differences in litter input and decomposition rates. Removing the litter could reduce the amount of C these crops can sequester, reducing their effectiveness as a low C energy alternative to fossil fuels.

The rate of decomposition is controlled by three main interacting factors: climatic variables (temperature, moisture), litter quality (total C, total N, lignin etc) and decomposer organism activity (fungi, bacteria, and invertebrates) (Singh & Gupta, 1977; Bardgett, 2005; Blair *et al*., 1990). The rate at which soil organisms can decompose litter is affected by the litter quality and climatic variables (Blair *et al*., 1990), so it has been argued that climate and litter quality are the most important factors in decomposition (Swift *et al*., 1979). Couteaux *et al*., (1995) found that in general, climate was the dominant factor in decomposition under unfavourable conditions, and litter quality was dominant in favourable conditions. The climatic variables that generally have the greatest effect on the rate of decomposition are temperature and precipitation or soil moisture, where higher temperatures and moisture contents lead to a higher rate of decomposition (Meentemeyer, 1978). The decomposition rate due to chemical composition is largely controlled by the relative proportion of C components, which can vary greatly between litters and breakdown at different rates. Generally there are three main C components: the labile soluble C fraction, which is easily broken down and includes free amino acids, organic acids and sugar, that are readily available to soil microbes; the middle fraction, which includes cellulose and hemicelluloses and are moderately labile; and the last fraction, which is

the most recalcitrant, including substances such as lignin (Bardgett, 2005). The chemical composition is used as a way to predict the rate of decomposition with decomposition negatively related to C:N ratios, lignin content and lignin:N and positively correlated with N concentrations (Melillo *et al*., 1982; Melillo *et al*., 1989) but this is not universal and generally depends greatly on plant species and environmental conditions.

Several studies have attempted to investigate the effects of litter quality and climatic variables using transplant experiments, where litter from different habitats is swapped to determine the main drivers of litter decomposition (Gholtz *et al*., 2000; Ayres *et al*., 2006). Some studies suggest that litter may decompose more rapidly in its home environment (Gholtz *et al*., 2000) but this is not always the case (Chapman & Koch, 2007). Where this is the case, transplant studies have called this a 'home field advantage' (HFA) linking it to soil microbes and fungi that are optimised to decompose a certain type of litter (Gholtz *et al*., 2000; Ayres *et al*., 2009)

Both *Miscanthus* and SRC willow have been studied extensively in terms of their suitability as bioenergy crops (i.e. yield capability) but there have been very few studies investigating soil processes in both these crops including the potential of these crops to sequester C through the accumulation of organic residues. Both of these crops produce a large amount of litter but little information is available about litter decomposition in these crops (Beuch *et al*., 2000; Amougou *et al*., 2011). The aim of this study was to investigate the C contribution to the soil surface from litter, determine litter decomposition rates, and establish the main drivers of decomposition in *Miscanthus* and SRC willow. This was done through quantifying litter input over the litterfall period; the rate of decomposition using the litter bag technique and measuring the associated litter chemistry; and by using a reciprocal swap experiment

to determine if decomposition was driven by environmental factors or litter chemistry. Several studies investigating turnover of SOM in *Miscanthus* and the effects of *Miscanthus* litter on earthworm communities have reported that *Miscanthus* litter has a high C:N ratio, in the range of 74 to 115 (Beuch *et al*., 2000; Foereid *et al*., 2004; Felten & Emmerling, 2011). Therefore it hypothesised that *Miscanthus* will have a slower rate of decomposition compared to SRC willow litter, which is reported to have a higher litter N content compared to *Miscanthus* and so a lower C:N ratio (Baldy *et al*., 1995). It is also hypothesised that due to the high C:N ratio of *Miscanthus* litter, that litter decomposition will be mostly determined by litter quality. Due to the lower C:N ratio of SRC willow litter but the high lignin content (27%) (Šlapokas & Granhall, 1991), it is hypothesised that decomposition will be effected by both litter quality and environmental parameters.

4.3 Material and Methods

4.3.1 Study Site and Experimental Design

The field study was conducted on a commercial farm in Lincolnshire UK, in adjacent fields of *Miscanthus* and SRC willow. The mean minimum temperature was 6.2°C (30-year mean 1980 to 2010) and mean maximum temperature of 13.4°C (30-year mean). The mean annual precipitation was 616 mm (30-year mean). The soil type is a fine clay loam with approximately 25, 29 and 49% clay, sand and silt content respectively, with further soil parameters for both crops described in Table 4.1.

Table 4.1 - Soil parameters for *Miscanthus* and SRC Willow at Lincoln, UK. Values are means determined from monthly soil cores (n=5, 0-15 cm depth) collected over study period (Nov 09 to Nov 10) with standard errors shown in brackets.

Soil Parameter	*Miscanthus*	Willow
Soil pH	7.1	5.6
Bulk Density (g cm^{-3})	1.41 (0.04)	1.33 (0.01)
Water-filled pore space (%)	71.72 (4.60)	67.12 (4.32)
Soil Moisture (%)	29.0 (1.13)	30.1 (1.00)
C content (%)	1.43 (0.04)	1.80 (0.06)
N content (%)	0.28 (0.01)	0.29 (0.01)

The SRC willow was established in 2000 and was planted at a density of approximately 15,000 stools ha^{-1} with different varieties to prevent disease spread across the plantation. The crop has been harvested in 2004, 2007 and 2011 with yields of 20, 26, and 19 t ha^{-1} respectively. No fertiliser or herbicide was applied during this study. The *Miscanthus* was established in 2006 at a density of 10,000 rhizomes ha^{-1}.

The crop has been harvested annually with yields of 5, 10 and 6 t ha^{-1} for 2009, 2010 and 2011 respectively. The only addition of fertiliser was in April 2010, when a PK fertiliser was applied at a rate of 125 kg ha^{-1}. The land management prior to land conversion to both energy crops was a three year crop rotation of wheat followed by one year of oilseed rape. Both fields had three years of wheat directly before conversion to energy crops.

In both crops, five sampling blocks (3 x 6 m) were established. In the SRC willow, sampling blocks were a minimum and maximum distance of 27 m 189 m apart respectively, and in the *Miscanthus*, the minimum and maximum distance between blocks was 50 m and 219 m respectively. In these blocks, equipment was set up to measure litterfall and litter decomposition (litter bags).

4.3.2 Litter Input Rates

Litter input rates were measured using two litter traps (52 x 42 x 9 cm) placed in each sampling block, in each crop from October 2008 to February 2011. Litter was collected from the traps once a month at the time of litter fall and dried at 30°C until a constant mass was reached. A subsample was taken and freeze-milled, to determine total C and N (TruSpec CN analyser, LECO, UK).

4.3.3 Litter Decomposition

Litter decomposition was measured over 12 months from November 2009 to November 2010 using a litter bag technique (Olson, 1963). Litterbags (20 x 10 cm) were made from a 1 mm nylon mesh (PlastOK, Ltd., UK) and were filled with approximately 5 g of air dried litter that was collected from the field in October 2008. *Miscanthus* litter was cut into 15 cm lengths before being placed into the bags. All

bags were then sealed using a using a heat sealer and then pinned to the soil surface using metal pins, in the litter layer. *Miscanthus* and SRC willow litterbags were placed in each sampling block in their native crop (hereafter referred to as M^{Home} or W^{Home}) and then removed after 1, 2, 4, 6, 8, 10, and 12 months (7 bags per sampling block, 35 per crop).

4.3.4 Reciprocal Swap Experiment

A reciprocal swap experiment was used to determine if litter quality or environmental effects were the main drivers of decomposition. This involved deploying litter bags in a non-native environment i.e. *Miscanthus* material in SRC willow and *vice versa*. The experimental approach was identical to the first experiment with 7 bags added to each of the five blocks for removal 1, 2, 4, 6, 8, 10 and 12 months after being deployed. These litterbags are referred to as M^{Away} and W^{Away} for *Miscanthus* and SRC willow respectively.

4.3.5 Processing Litter Material and Chemical Analysis

On retrieval from the field, litter material was removed from the bags and cleaned using water to remove any soil attached to the litter. After cleaning, mass loss was determined by drying litter samples at 80°C for 24 hours. A sub-sample was taken and freeze-milled for determination of total C and N, where C and N release was expressed as nutrient loss. Mass loss was expressed as a function of initial dry mass after taking into account mass loss due to travel and the initial air-dried status of the litter. Fibre, cellulose, and lignin fractions were determined on sub-samples taken from initial litter and 12-month litter bags using acid-detergent fibre sulphuric acid procedure as described in Rowland and Roberts, (1994). Briefly, fibre was determined

as the fraction remaining after treating the litter with boiling acid detergent to hydrolyse protein. The cellulose fraction was determined by treating the fibre fraction with 72% sulphuric acid to destroy cellulose and was the difference between initial and remaining fractions. Finally, the residue was ignited at 550°C for 2 hours to destroy all remaining organic material and the lignin fraction was the difference between pre- and post-ignition.

4.3.6 Field and Soil Parameters

Air and soil temperature were measured using a Tiny Tag temperature logger with integral stab probe (0-7 cm depth) (Gemini Data Loggers, UK). Volumetric soil moisture content (0-6 cm) was determined using a ML2x Theta Probe and Meter HH2 (Delta T Devices, UK) and taking the mean of three measurements from each sampling block. Continuous measurements of precipitation (203 mm diameter automated tipping rain gauge, Rimco 8500) were made from an automatic meteorological station close to both fields. Gravimetric soil moisture was determined from subsamples taken from fresh soil samples (0-15 cm depth, 5 cm diameter) taken from within each block, that were oven dried at 105°C for 24 hours.

4.3.7 Data Analysis

All statistical analysis was done using R (version 2.14.0). Normality of variables was tested using Shapiro-Wilk test and values were log transformed when appropriate. The annual decomposition (k) was determined using the exponential decay model [1], proposed by Olson (1963). Here X_0 is the original mass of litter, X_t is the mass remaining at time t and t is time (years).

$$k = \ln(X_0/X_t)/t \quad [1]$$

C, N, fibre, cellulose and lignin release/loss were determined using the method used by Bragazza *et al*., (2007), using [2]. Where X_0 is the mean nutrient concentration (mg g^{-1}) of plant litter before burial, X_1 is the nutrient concentration in the litter bag after one year of burial, W_0 is the mass of plant litter in the bag before burial and W_1 refers to the mass of the same content after 1 year.

$$\text{Nutrient release (\%)} = ((X_0 W_0 - X_1 W_1) / (X_0 W_0)) \times 100 \quad [2]$$

The 'home field advantage index' (HFAI) was determined using the method proposed by Ayres *et al*., (2009). This gives the net value of the percent faster (or slower) mass loss, for both species used in the experiment, of litter when it decomposes at 'home' compared to 'away'. Where 'A' and 'B' are the species used in the experiment and 'a' and 'b' are their respective habitats, then the relative mass loss within each habitat is expressed in [3] and the percent HFA for both species is calculated using [4].

$$A_{RMLa} = 100 \times (A_a / (A_a + B_a)) \quad [3]$$

$$HFAI = 100 \times (((A_{RMLa} + B_{RMLb}) / 2)) / ((A_{RMLb} + B_{RMLa}) / 2))) - 100 \quad [4]$$

Significance of differences in mass loss, k values, C and N release at certain time points were determined using a two-way ANOVA using type 3 sums of square due to unequal sample sizes and a pair-wise comparison was used to determine difference between treatments when a significant difference was found. A linear mixed effects

model, with ‘site’ as random error, was used to determine if single parameters (litter quality and environmental) were significant in mass loss. The linear mixed effects model was also used with all significant parameters for each treatment to determine the overall parameter(s) that were significant for mass loss. Parameters were removed from the model if they were not significant to mass loss, leaving only significant parameters. Significance was accepted when $p<0.05$.

4.4 Results

The experimental fields were adjacent to each other and had the same underlying soil conditions. Both fields had similar soil conditions with respect to soil moisture, water-filled pore space and N content but differed in pH, bulk density (BD) and C content, with willow having lower pH, lower BD, and higher soil C content than the *Miscanthus* field (Table 4.1).

4.4.1 Litter Input

Litterfall was measured in both *Miscanthus* and SRC willow from October 2008 to February 2011 (Figure 4.1).

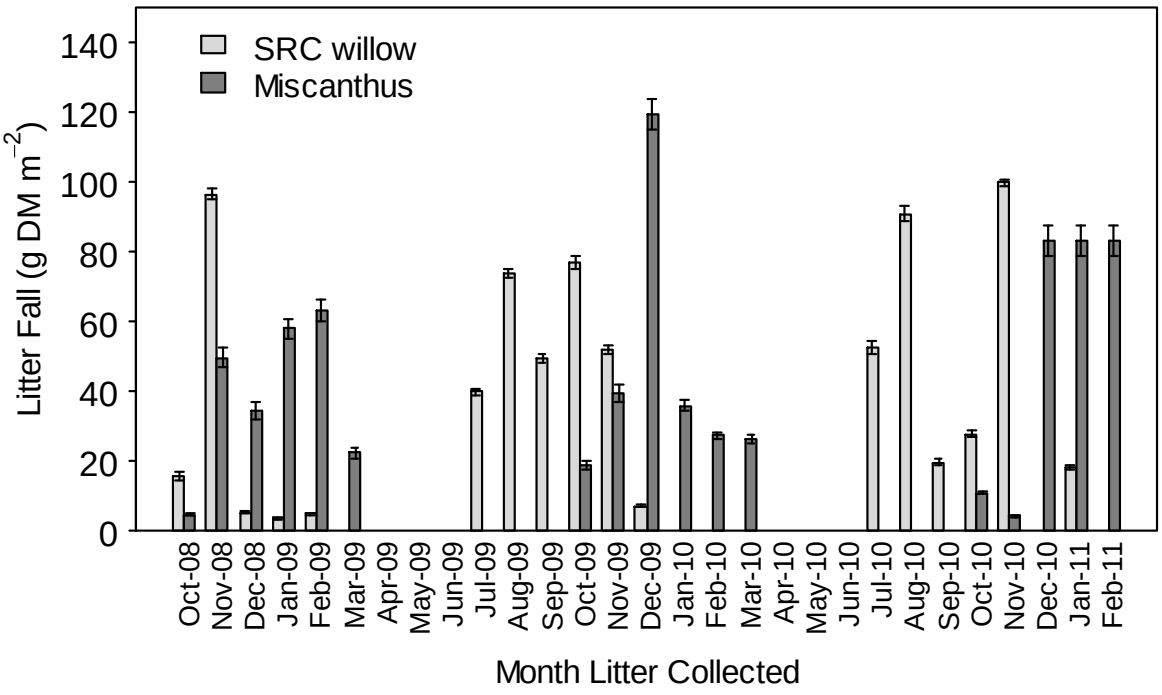

Figure 4.1 - Litter fall (g dry mass (DM) m^{-2}) for *Miscanthus* and SRC willow from October 2008 to February 2011. Values are monthly means (n=10) and error bars represent standard error values. Access to the crop was limited due to crop collapse under snow from December 2010 to February2011. The values for these months is an equal split of the total litter collected in February 2011 when access to the crop could be gained, though it is not possible to say when the litter actually fell.

Miscanthus had three defined litterfall periods (LFP) within this time, and litter collection began in October and ended in the spring. The litterfall dynamics varied between LFP, with LFP 1 (Oct-08 to Mar-09) litterfall ranging from 4.3 to 63 g DM m^{-2} and LFP 2 (Oct-09 to Mar-11) litterfall ranging from 18.3 to 119.1 g DM m^{-2}, with a peak of litterfall in December 09, unlike LFP 1. The litterfall range in LFP 3 (Oct-10 to Mar-11) is difficult to determine since heavy snow caused the *Miscanthus* to collapse and interlink making the field-site inaccessible during December and January, so the February litter collection was assumed to be a cumulative total from December 2010 and that was split equally between the months. Despite the variation in the range of litterfall across each LFP, the total litterfall over each LFP was remarkably similar, ranging from 2.3 to 2.7 t ha^{-1} (Table 4.2). This was more surprising given the different yields between years. The lower total litterfall in LFP 1 could be attributed to the age of the crop, since the crop was only in its third growing season and still in the establishment phase. The total C and N input into the soil was different between LFP, with LFP 1 litter having higher N and lower C content than LFP 2 (Table 4.2).

SRC willow also had three distinct LFP, but the LFP varied in length and start date unlike *Miscanthus*. LFP 1 litterfall started in October 2008 and lasted for 5 months with litterfall ranging from 3.4 to 96.1 g DM m^{-2}. LFP 1 litterfall dynamics varied from the other LFP by having a peak of litterfall in November 2008 and then very little litterfall thereafter. Both LFP 2 and 3 litterfall started in July but lasted approximately 6 months, with litterfall for LFP 2 ranging from 6.9 to 76.6 g DM m^{-2} and LFP 3 litterfall ranging from 17.7 to 99.6 g DM m^{-2}. The total litterfall per LFP also varied between LFP, with the total litterfall increasing with LFP from 1.25 to 3.07 t ha^{-1} (Table 4.2). LFP 1 had less than half the total litterfall compared with the

other two LFP, which could be attributed to missing the beginning of the season or it may be that there was less litterfall since this was the first growing season after the harvest in 2008.

The C and N content of the litter for both *Miscanthus* and SRC willow did not vary greatly over each litter fall period and are not shown.

Table 4.2 - Total dry mass and C and N contents and C:N ratio for each litter fall period from *Miscanthus* and SRC willow at a site in Lincolnshire, UK. Dry mass is the sum of litterfall over a given litter fall period. C and N values are the mean values ± standard error values over the litterfall period, expressed as a function of dry mass. nd is not determined.

	Total Dry Mass ($kg\ ha^{-1}$)	N ($kg\ ha^{-1}$)	C ($kg\ ha^{-1}$)	C:N Ratio
Willow				
LFP 1: Oct-08 to Feb-09	1245.0 ± 0.7	18.4 ± 0.05	579.9 ± 0.17	31.6 ± 0.93
LFP2: Jul-09 to Dec-10	2974.0 ± 1.2	44.6 ± 0.04	1414.1 ± 0.55	31.7 ± 1.16
LFP3: Jul-10 to Nov-10	3071.0 ± 1.2	45.5 ± 0.08	1383.1 ± 0.56	30.8 ± 1.70
Miscanthus				
LFP 1: Oct-08 to Mar-09	2304.0 ± 2.1	26.7 ± 0.24	983.8 ± 0.25	45.5 ± 2.42
LFP 2: Oct-09 to Mar-10	2654.0 ± 1.9	18.6 ± 0.20	1124.5 ± 0.60	74.4 ± 1.26
LFP 3: Oct-10 to Feb-11	2630.0 ± 4.5	nd	nd	nd

4.4.2 M^{HOME} and W^{HOME} Experiment

4.4.2.1 Litter Decomposition

Both *Miscanthus* and SRC willow litter had a surprisingly similar mass loss of about 40% after 12 months (Figure 4.2a). Although total losses between crops were similar, the dynamics of mass loss varied between the two crops over the 12-month period of measurements. SRC willow had a high mass loss in the first month (18%) unlike *Miscanthus* (6%) and then had a steady mass loss over the remainder of the study, with a 12-month mass loss of 38.8%. *Miscanthus* had a steady mass loss over the first 10 months of the study and then highest mass loss was found from month 10 to 12, with a final mass loss after 12 months of 41.8%. This mass loss dynamic is highlighted further by the decomposition rates (k) in Figure 4.2b, showing SRC willow to have significantly (ANOVA, $p<0.05$) higher decomposition rates than *Miscanthus* for the first two months and then both crops having similar decomposition rates thereafter, with only 6 months showing a significant difference in decomposition rate (ANOVA, $p<0.05$). After 12 months, the decomposition rates between SRC willow and *Miscanthus* were not significantly different with decomposition rates of 0.49 $year^{-1}$ and 0.54 $year^{-1}$ respectively.

4.4.2.2 Litter Chemistry

Initial litter chemistry (Table 4.3) showed that the N and C content of SRC willow litter was significantly higher (t-test, $p<0.05$) than that of *Miscanthus* litter, most notably the N content of SRC willow (1.2%) was more than twice that of *Miscanthus* litter (0.54%). *Miscanthus* litter had a high C:N ratio of 81 and was significantly higher than that of the C:N ratio for willow litter (37).

Table 4.3 - Initial litter chemistry (% of dry mass) used in 12-month decomposition study in Lincoln, UK. Values are means (n=5) ± standard error. Litter characteristics within litter that have the same letter are not significantly different (t-test).

Litter Characteristic	*Miscanthus*	SRC Willow
N (%)	0.54 ± 0.04^{b}	1.20 ± 0.01^{a}
C (%)	42.41 ± 0.77^{b}	44.62 ± 0.16^{a}
C:N ratio	80.77 ± 6.38^{a}	37.22 ± 0.18^{b}
Fibre (%)	65.56 ± 1.41^{a}	62.76 ± 1.07^{a}
Cellulose (%)	48.12 ± 2.80^{a}	23.08 ± 0.48^{b}
Lignin (%)	12.58 ± 1.17^{b}	39.71 ± 0.75^{a}

The release of C and N from both SRC willow and *Miscanthus* litter over the study period can be seen in Figure 4.3. Both M^{HOME} and W^{HOME} showed a steady release of C and N over the 12-month study period. In all but two months (4 and 6) M^{HOME} showed a significantly higher (ANOVA, $p<0.05$) release of N than W^{HOME}. M^{HOME} had a high release of N in the first month (42%) but there was little N release until month 8, 10 and 12, which showed 48, 51 and 62% release respectively (Figure 4.3a). W^{HOME} had the highest release in N in the first month (29%) and then showed slow but steady release of N thereafter, with 42% release after 12 months. W^{HOME} showed significantly higher (ANOVA, $p<0.05$) C release than M^{HOME} in all but two months, 10 and 12. M^{HOME} showed the highest release of C in months 10 and 12, with a final C release of 67% after 12 months (Figure 4.3b). W^{HOME} showed the highest release in C in the first month (29%) and like N release, showed a steady release of C thereafter with 62% release after 12 months. M^{HOME} had a high initial C:N ratio compared to W^{HOME}, which increased in the first month to correspond with high N release in the first month (Figure 4.3c). C:N ratio generally followed the pattern of mass remaining in M^{HOME}, with a drop in C:N ratio between months 10 and 12, corresponding with a

higher release of C and N between these months. W^{HOME} showed a steady drop in the C:N ratio over the study period from 37.2 to 24.5, generally following the pattern of mass remaining. This implies that C and N release occurred at similar rates throughout the study period, but with slightly higher C release than N, causing the decrease in C:N ratio.

The analysis of initial litter material for fibre, cellulose, and lignin can be seen in Table 4.3. Initial fibre concentrations were significantly different between M^{Home} and W^{Home} (63 and 66% respectively), where as M^{Home} had higher cellulose and lower lignin content than W^{Home} which were found to be significantly different (ANOVA, $p<0.05$). Loss of fibre, cellulose, and lignin content can be seen in Figure 4.4. After 12 months, there was a significant difference between M^{Home} and W^{Home} in fibre loss, with M^{Home} losing most overall (48%). There was no significant difference between M^{Home} and W^{Home} treatments with both showing around 60% reduction in cellulose content after 12 months. There was no significant difference in the lignin reduction between M^{Home} and W^{Home}, with loss of 34% and 30% lignin respectively.

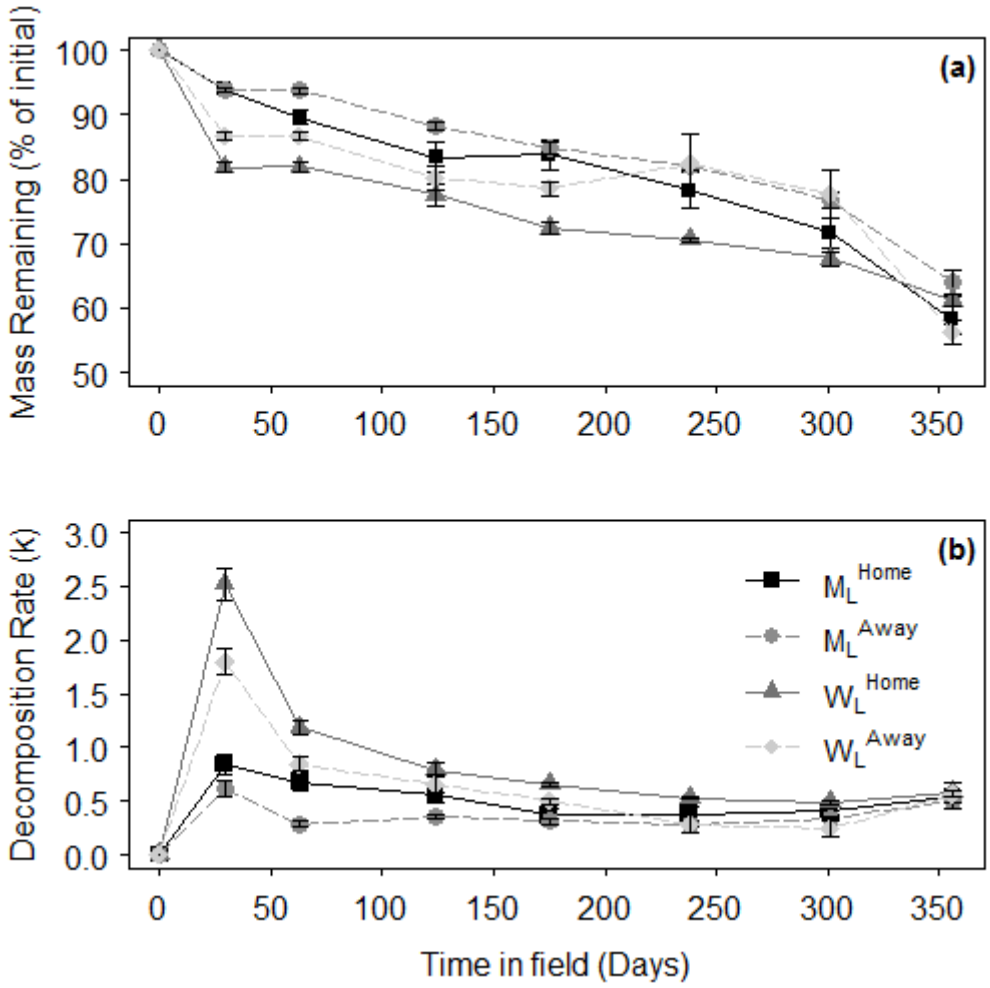

Figure 4.2 - Litter monthly means (n=5) of **a)** mass remaining (% of initial) and **b)** decomposition rate (k) for M^{Home}, W^{Home}, M^{Away} and W^{Away} at each time bags were removed from the field. Error bars represent standard error values.

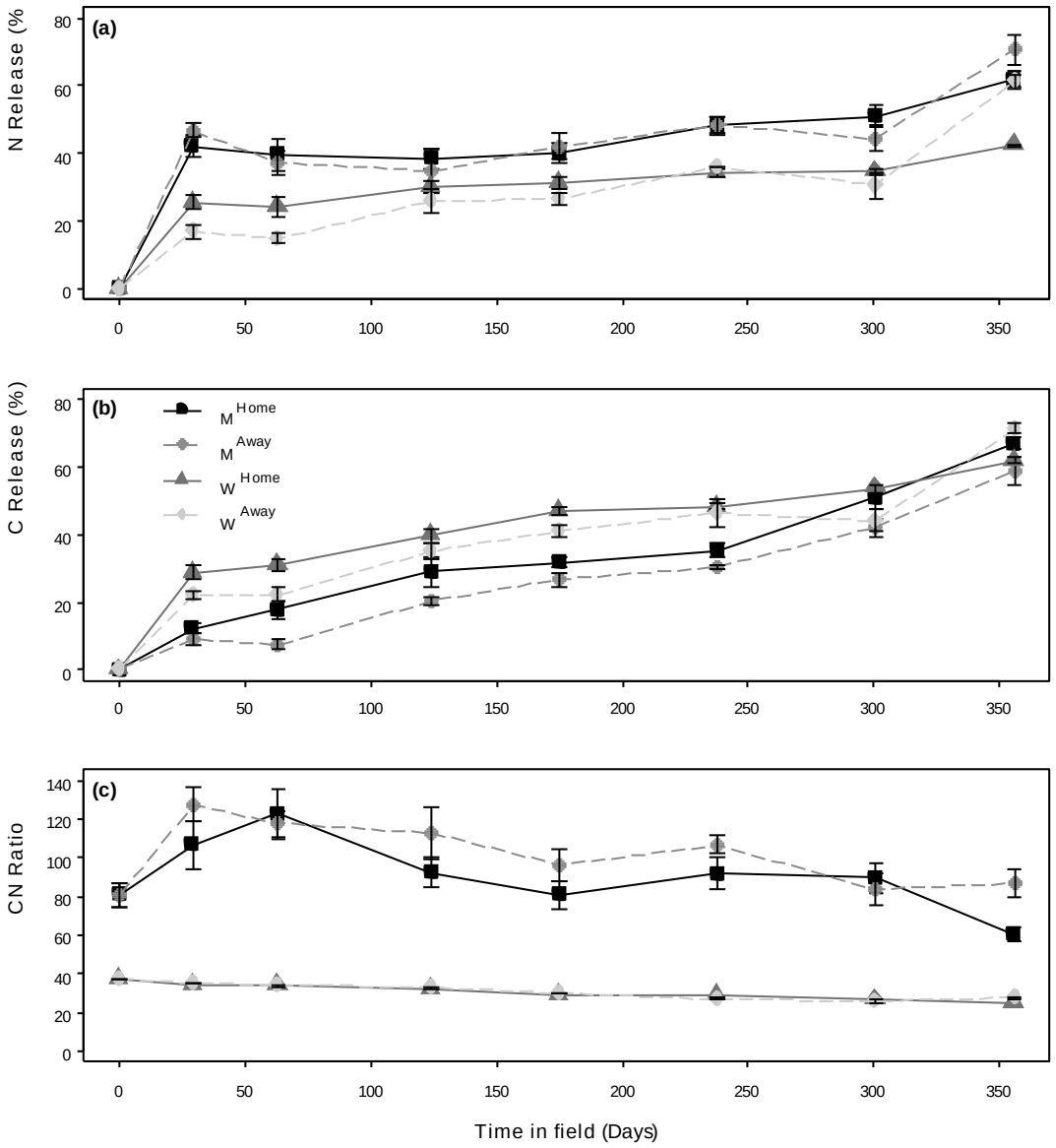

Figure 4.3 - Mean monthly values (n=5) for **a)** N release and **b)** C release and **c)** C:N ratio for all litterbags for M^{Home}, W^{Home}, M^{Away} and W^{Away} at each time bags were removed from the field. Error bars represent standard error values.

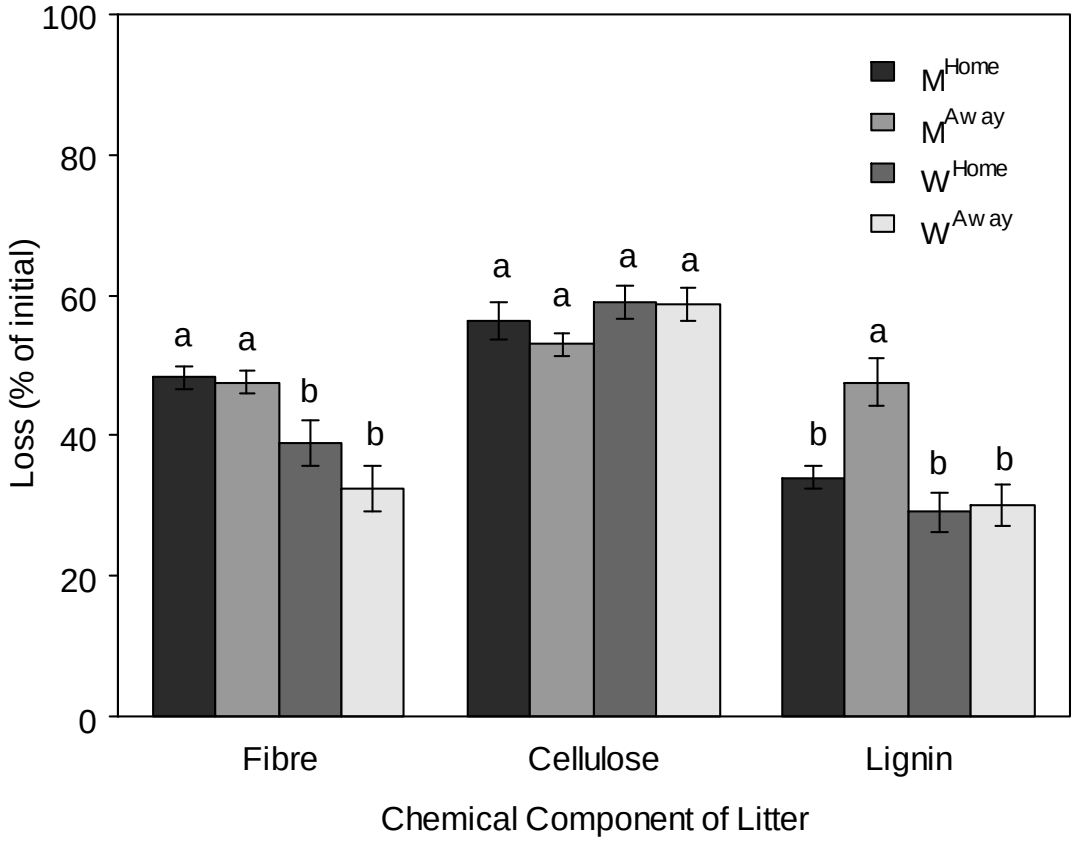

Figure 4.4 - Mean Percentage loss (± SE) of fibre, Cellulose and Lignin, expressed as % of initial total content, from all treatments after 12 months in the field. Significant differences (ANOVA and pair-wise post-hoc comparisons; $p<0.05$) between treatments for fibre, cellulose and lignin are indicated by different letters.

4.4.3 Reciprocal Swap Experiment

4.4.3.1 Litter Decomposition

Miscanthus litter treatments, M^{HOME} and M^{AWAY}, showed similar mass loss dynamics, with M^{AWAY} generally showing a lower mass loss but this was only significant in month 2 of the study (ANOVA, $p<0.05$) (Figure 4.2a). There was little mass loss in the first month of the study with approximately 6% loss for both M^{HOME} and M^{AWAY}. In month 2, M^{AWAY} showed little mass loss compared to M^{HOME} and this was also reflected in the decomposition rate (k) (Figure 4.2b). The bulk of the mass loss came after 6 months with a total of 41.7% and 36.1% loss, and decomposition rates of 0.54 and 0.43 $year^{-1}$ for M^{HOME} and M^{AWAY} respectively, after 12 month (Figure 4.2b). Willow treatments, W^{HOME} and W^{AWAY}, showed a high mass loss (18% and 13% respectively) in the first month, with high decomposition rates of 2.41 and 1.72 $year^{-1}$ respectively, which were significantly different (ANOVA, $p<0.05$) (Figure 4.2ab). After two months, decomposition rates were slower (below $k=1.0$ $year^{-1}$) and remained relatively constant to the end of the study, with only months 6 and 8 showing a significant difference in mass remaining and decomposition rates. W^{AWAY} had lower mass loss than W^{HOME} except at 12 months, when W^{AWAY} showed a sharp increase in the rate of decomposition from 0.32 to 0.57 $year^{-1}$ from months 10 and 12 respectively.

3.4.3.2 Litter Chemistry

Miscanthus treatments, M^{HOME} and M^{AWAY} showed very similar N release dynamics over the 12 month study, with only significant difference between treatments at 12 months (ANOVA, $p<0.05$) (Figure 4.3a). For the majority of the study N release stayed at around 40%, for both treatments (i.e. little further release of N) and only

increased to 62% and 71% for M^{HOME} and M^{AWAY} at 12 months. The C release dynamics between treatments varied over the 12-month study, with months 2, 4, and 6 showing a significant difference between treatments (ANOVA, $p<0.05$) (Figure 4.3b). M^{HOME} and M^{AWAY} showed a steady release of C over 12 months, with M^{HOME} generally having higher C release. Total C release after 12 months was 67% and 59% for M^{HOME} and M^{AWAY} respectively. The C:N ratio for M^{AWAY} was generally higher than M^{HOME}, but this was only significant in the last month of the study (Figure 4.3b). The C:N ratio generally followed the pattern of litter mass remaining. In terms of other litter chemistry, no significant difference was found between treatments for fibre (48%) or cellulose (55%) loss, however, M^{AWAY} showed a significantly higher (ANOVA, $p<0.05$) loss of lignin (48% and 34% respectively) than M^{HOME} (Figure 4.4).

Willow treatments, W^{HOME} and W^{AWAY} showed very similar N release dynamics over the 12 month study, with significant difference between treatments only found at 2 and 12 months (ANOVA, $p<0.05$) (Figure 4.3a). There was a slow release of N from approximately 20% to 32% from 1 to 10 months but this increased to 61% for W^{AWAY} compared to 42% for W^{HOME} at 12 months. The C release dynamics were also very similar between treatments, with just months 1 and 2 showing a significant difference between treatments (ANOVA, $p<0.05$) (Figure 4.3b). W^{WOME} and W^{AWAY} showed a steady release of C over 12 months, with W^{AWAY} generally having higher C release. Total C release after 12 months was 62% and 71% for W^{AWAY} and W^{AWAY} respectively. The C:N ratio of W^{HOME} followed an almost identical pattern, with a steady reduction in C:N ratio throughout the study (Figure 4.3c). When examining other litter chemistry, there were no significant differences between treatments for fibre (36%), cellulose (59%), or lignin (30%) loss (Figure 4.4).

3.4.3.3 Decomposition Drivers

Litter quality and environmental drivers can both control decomposition, and linear mixed models were used to highlight the relative importance of these drivers (Table 4.4). It was clear that in all treatments litter C content was highly important to the rate of litter decomposition as highlighted by large F values, and it was included in all final linear regressions for mass loss. Precipitation was not significant in litter decomposition for any of the treatments.

For the *Miscanthus* treatments M^{HOME} and M^{AWAY}, temperature (soil and air), litter C, and litter C:N ratio were common significant parameters in mass loss (Table 4.4). The results highlighted difference between the two treatments with litter N significant in M^{HOME} decomposition but not M^{AWAY} and soil moisture (0-6 cm depth) was significant for M^{AWAY} but not M^{HOME}. The final linear regression analysis showed that Litter C was an important parameter in decomposition for both *Miscanthus* treatments, but that air temperature was also significant for M^{AWAY} decomposition.

Common significant parameters for willow (W^{HOME} and W^{AWAY}) were temperature (soil and air), soil moisture (0-6 cm and 0-15 cm depth), litter C, and litter C:N ratio (Table 4.4). Litter N was found to be significant for W^{AWAY} but not for W^{HOME}. The regression analysis highlighted that soil temperature and litter C were the most important parameters in the rate of decomposition for W^{HOME} but for W^{AWAY} litter C, and litter N were important.

To determine if litter decomposed faster (or slower) in its native environment compared to its non-native environment, the formula by Ayres *et al*., (2009) was used to calculate the home field advantage index (HFAI), giving a single value for both crops. Litter showed that after 6 months decomposition there was a net HFA for mass

loss of 16.7%, however after 12 months, there was only a minor HFA of 1.3% for mass loss.

Table 4.4 - Results of a linear mixed model for litter mass loss (% of initial) as influenced by climatic variables and leaf chemistry. Values include the F statistic, degrees of freedom (df) and significant levels (accepted when $p<0.05$) for each litter treatment. The significant parameters for each treatment were used in a mixed effects model to determine the most significant parameters in litter decomposition.

Variable	M^{HOME}			M^{AWAY} †			W^{HOME}			W^{AWAY} †		
	F	df	p	F	df	p	F	df	p	F	df	p
Air Temp	12.7	20	0.002	59.7	19	0.0001	27.5	23	0.0001	17.9	22	0.0003
Soil Temp	7.6	20	0.012	53.3	19	0.0001	44.3	23	0.0001	25.9	22	0.0001
T Moisture	1.6	20	0.221	23.6	19	0.0004	19.4	23	0.0004	11.3	22	0.003
G Moisture	1.0	20	0.326	3.2	19	0.088	19.0	23	0.0004	10.4	22	0.004
Precip	0.1	20	0.751	0.4	19	0.545	0.3	23	0.622	4.3	22	0.049
Litter N	6.4	20	0.020	0.2	19	0.708	4.5	23	0.045	72.5	22	0.0001
Litter C	341.2	20	0.0001	170.2	19	0.0001	496.2	23	0.0001	298.2	22	0.0001
Litter CN	7.5	20	0.013	14.7	19	0.001	157.5	23	0.0001	42.2	22	0.0001
Final	ML = Litter C			ML† = Air Temp + Litter C			ML = Soil temp x Litter C			ML† = Litter C + Litter N		

Tmoisture is soil moisture at 0-6 cm depth taken from Theta probe measurements
Gmoisture is gravimetric soil moisture at 0-15 cm depth.
Precip is precipitation from 7 days before sampling
ML = mass loss
† indicates that mass loss was log transformed

4.5. Discussion

4.5.1 Quantifying Litterfall

The aim of this part of the study was to quantify litter input, litterfall dynamics and litter chemistry in both of these crops. The mean litterfall over the study was 2.53 and 3.02 t ha^{-1} (assumed full LFP only) for *Miscanthus* and SRC willow respectively. For *Miscanthus*, this is broadly supported by other studies, which suggest that litterfall is over 2 t ha^{-1} (Christian & Riche, 1998; Amougou *et al*., 2011), but other studies report much higher litterfall, between 4 and 8 t ha^{-1} (Himken *et al*., 1997; Hanson *et al*., 2004). The discrepancy in results is likely to be a result of different ages of crops being used, and those studies reporting higher litterfall were generally in older crops. For SRC willow the results in this study seem to be similar to other studies findings, in the range of 2.47 to 5.3 t ha^{-1} (Jonczak & Czarnecki, 2008; Vandecasteele *et al*., 2009).

For *Miscanthus* the litterfall dynamics across LFP 1, 2, and 3 were different but this had little effect on the total litterfall between seasons (2.3 to 2.7 t h^{-1}). This was a little surprising since the yields for the crop did vary between years, but may indicate that litterfall dynamics are influenced by climatic conditions, which were similar between years (Valenti *et al*., 2008). SRC willow litterfall dynamics behaved differently, and the first season showed a delay to the litterfall period and a lower total litterfall compared to the other seasons. This could be due to the timing of the experiment not capturing the start of the litterfall period, since subsequent seasons started in July, but it could be related to the fact that the crop was in its first season post harvest and the crop was smaller than in subsequent years. The total C and N content in both *Miscanthus* and SRC willow litter did not vary significantly over the litterfall period and this is likely due to the remobilisation of nutrients to the perennial part of the plant

(for both *Miscanthus* and SRC willow), with the rest staying in the dead litter (Beale & Long, 1997; Berg & McClaugherty, 2008; Strullu *et al*., 2011).

4.5.2 Quantifying Litter Decomposition

The second aim of the study was to measure decomposition of *Miscanthus* and SRC willow litter, and to investigate litter chemistry. It was hypothesised that due to the high initial C:N content of *Miscanthus* litter, that the rate of decomposition would be slower than SRC willow. Surprisingly, the total mass loss over the year experiment was the same for *Miscanthus* and SRC willow (40%) but the dynamics of litter decomposition between the crops over the study were different. This was attributed to the high lignin content of the SRC willow litter, which slowed the rate of decomposition in the later stages of the experiment. Therefore, SRC willow litter decomposition was more comparable with *Miscanthus* decomposition rates later in the experiment.

For SRC willow only one other comparable study was identified from Šlapokas and Granhall (1991) who found approximately 20% mass loss after 6 months, equivalent to 40% after 12 months, which is comparable with the results in this study. For *Miscanthus* no other studies were found that measured litter decomposition in the field but our results were similar to other studies investigating litter decomposition with high a C:N ratio. Cortet *et al*., (2006) reported 25 to 40% mass loss from maize after 150 days (C:N ratio 60), and Collins *et al*., (1989) found 33% mass loss from wheat residue after 377 days in the field. The main difference in the mass loss dynamics of the two crops came in the first two months, with SRC willow having significantly higher mass loss than *Miscanthus*. This may be attributed to a higher soluble C content in the SRC willow, and higher precipitation in these months inducing leaching

(Šlapokas and Granhall, 1991). Šlapokas and Granhall (1991) reported high soluble C levels in willow of between 29% and 34% and our results fit within this range with approximately 30% C release in the first month (Figure 4.3). The low litter decomposition rate for *Miscanthus* in the first few months is due to the high C:N ratio of the litter and this has been known to reduce decomposition rates by inhibiting microbial activities through lack of available N (Christensen, 1985; Cortet *et al*., 2006; Zhang *et al*., 2008). The low initial N content of *Miscanthus* litter may also have resulted in a lower soluble C fraction (Reinertsen *et al*., 1984); Recous *et al*., 2005) also reducing the decomposition rate. A low soluble C fraction in *Miscanthus* litter has been found in other studies, reporting approximately 7% soluble fraction (Le Guillou *et al*., 2001; Luxhøi *et al*., 2002; Magid *et al*., 2004). This however, is not consistent with other findings that report had a much higher soluble C content in the range of 22 to 36% (Kohli *et al*., 1999; Amougou *et al*., 2011). Since the soil N content was also low, this may have compounded the lack of available N for decomposer organisms also causing slow decomposition rates (Recous *et al*., 1995). The mass loss in the latter stages is likely to have been slowed by the initial high cellulose and lignin content, especially in SRC willow. High lignin content in *Miscanthus* litter has be shown to negatively impacted litter decomposition (Dresbøll & Magid, 2006; Amougou *et al*., 2011) and has been found in other studies (Melillo & Aber, 1982).

4.5.3. Reciprocal Swap Experiment

The purpose of the reciprocal swap experiment was to determine if litter quality or environmental parameters were the main driver of litter decomposition in *Miscanthus* and SRC willow. It was hypothesised that due to high C:N ratio of *Miscanthus* litter,

that decomposition would be mostly controlled by litter quality, but SRC willow decomposition would be controlled by both litter quality and environmental factors due to the lower C:N ratio compared to *Miscanthus,* and the high lignin content. The results would suggest that litter quality was the main driver of decomposition in both crops, with Litter C, and C:N ratio important for *Miscanthus* decomposition, and Litter C, and N, and C:N ratio important for SRC willow decomposition. Environmental effects also influenced decomposition but were secondary to litter quality more so in SRC willow than *Miscanthus*.

There was a significant difference in decomposition rates between W^{HOME} and W^{AWAY} in the first few months of the study. This may be as a result of the different understory environments between the two crops, and the SRC willow soil surface being covered by moss. Moss has shown to increase moisture levels and increase mass loss in other experiments (Garcia-Pausas *et al*., 2004) and may be a possible cause for the differences in the mass loss in the first months of the study. There were significant differences in mass loss at other stages (6 and 8 months) in the study between W^{HOME} and W^{AWAY}, which suggests that although litter quality was highly significant in mass loss, that environmental parameters are also important in determining mass loss in SRC willow.

M^{AWAY} showed very similar mass loss dynamics to M^{HOME} throughout the study suggesting that litter decomposition was mostly driven by litter quality and especially litter C content, characterised by high lignin content and low N content (Recous *et al*., 1995; Melillo & Aber, 1982). There were differences in the significance of parameters important to litter decomposition, most notably soil moisture, which was significant in M^{AWAY} but not for M^{HOME}. This may be related to the moss understory environment increasing soil moisture (Garcia-Pausas *et al*., 2004), but increased soil moisture did

not appear to have a significant difference on mass loss between treatments as there was no significant difference in mass loss after month 2 of the study. M^{AWAY} showed a significant difference in lignin loss after 12 months from all other treatments. This may be caused by soil organisms preferentially choosing to decompose *Miscanthus* litter due to its lower lignin content than SRC willow litter (Ayres *et al*., 2006).

Many studies have reported higher decomposition rates when litter decomposes in its native environment (HFA) (Gholtz *et al*., 2000; Ayres *et al*., 2009) and the results here support this, although we only found a slight HFA of 1% after 12 months. The HFA was much more pronounced after 6 months (17%) suggesting that the 'home' environment is more important to the earlier stages of decomposition in these crops and that litter quality becomes more important as the soluble and middle fraction are decomposed and the lignin fraction is left (Berg *et al*., 2008).

4.5.4. C Inputs

The information collected in this investigation allows the estimation the how much of litter C contributes to overall C stocks. Amougou *et al*., (2011) suggest that 53% of added C was mineralised in a 263 day laboratory experiment (47% stabilised). Given that this study suggests that litter contains 1.1 t C ha^{-1} y^{-1}, the annual contribution to soil C would be 0.52 t C ha^{-1} y^{-1}, suggesting that over the 20-year life span of *Miscanthus* the contribution from senescent litter would be 10.4 t C ha^{-1}. These values are reasonable given comparisons to studies suggesting *Miscanthus* contribution to total soil C sequestration of 0.66 t C ha^{-1} (Don *et al*., 2011) and in the range of 0.49 to 0.73 t C ha^{-1} y^{-1} (King *et al*., 2004). No specific C mineralisation rates could be found for SRC willow, so if it is assumed to be the same as *Miscanthus*, from an annual litter input of 1.1 t C ha^{-1} the contribution from senescent litter would be 0.52 t C ha^{-1} y^{-1}

and 10.4 t C ha^{-1} over a 20 year life span. The true mineralisation rate is likely to be lower due to the lack of disturbance (3-year harvest cycle) reducing mineralisation rates (Holland & Coleman, 1987). This would provide a reasonable explanation for the difference between our results and those predicted by Don *et al*. (2011) of 0.44 t C ha^{-1} y^{-1} for total C sequestration. Our results are in line with estimates suggested by King *et al*., (2004) who suggest that SRC willow could increase soil organic carbon by 0.55 to 0.83 t ha^{-1} y^{-1} and are high due to this range being inclusive of all organic C inputs.

4.6 Conclusion

The results from this study indicate that the rate of litter decomposition was mostly controlled by litter chemistry, owing to the high C:N ratio of *Miscanthus* and the high lignin content of SRC willow. The slow decomposition rate leads to the accumulation of litter on the soil surface, which is beneficial for soil C sequestration. Overall, our results show the importance of litter returning nutrients to the soil and C sequestration, and removing the litter could reduce the amount of C these crops can sequester, reducing their effectiveness as a low C energy alternative to fossil fuels.

4.7 Acknowledgements

The authors would like to thank everyone that helped with fieldwork including Simon Oakley and Sean Case from CEH at Lancaster. Special thanks to Clive woods and Helen Grant for help with litter chemistry analysis and allowing access to laboratory equipment. The work was funded by NERC and could not have been carried out without the generosity of the farmer, Jonathan Wright, who allowed us access to the field site.

Chapter 5

Partitioning soil respiration in the bioenergy crop *Miscanthus x giganteus* using a litter and root manipulation experiment.

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Declaration of contribution

I declare that this chapter consists of original work undertaken by myself. The contribution of co-authors is as such. Niall McNamara assisted with fieldwork and Jon Finch provided continuous environmental data (temperature and soil moisture) from a nearby meteorological station, Andy Stott analysed gas and soil samples for $\delta^{13}C$ and provided the isotope methods in this chapter. Niall McNamara, Jon Finch and Pete Smith provided PhD supervision and comments on this manuscript.

5. Partitioning soil respiration in *Miscanthus*

5.1 Abstract

A study was conducted in a *Miscanthus x giganteus* plantation in Lincolnshire, UK in order to partition total soil respiration into leaf litter respiration (R_L), rhizosphere respiration (root, rhizome and associated respiration from rhizodeposits) (R_R) and decomposition of soil organic matter (R_{SOM}). *Miscanthus* is a bioenergy crop used as a low C alternative to fossil fuels and one that has the potential to sequester C in soils. Partitioning soil respiration in this crop is key to understanding the processes behind soil respiration and future changes in C balance. Litter was added or removed from plots and live roots were removed from plots via trenching to determine the sources of respiration. Annual CO_2 flux from control plots ranged from 181 to 300 g C m^{-1} yr^{-1} in 2010 and 2009 respectively and R_L, R_R and R_{SOM} contributed, on average, 14%, 49%, and 37% respectively. These values are comparable with other reported values, although R_L fell below other reported values in 2009, which was attributed to low soil moisture, limiting the rate of decomposition. In addition, there was little evidence of priming from plots receiving double the amount of litter and this was attributed to the high C:N ratio of litter and low available N for microbial decomposition. The latter finding is of importance to bioenergy crops as it suggests that increases in net primary productivity could lead to increased belowground storage of C, but it is acknowledged that more evidence in needed.

5.2 Introduction

Soils are estimated to contain 2,500 Gt carbon (C), making them the largest pool of C in terrestrial ecosystems, totalling more than the atmospheric pool (760 Gt) and the biotic pool (560 Gt) combined (Lal, 2004). On a global scale, soil respiration is estimated to be in the range if 79 to 82 Gt C (Raich *et al*., 2002), making it the second largest C flux after photosynthesis and a key component of the global C balance (Schimel, 1995). Rates of soil respiration are highly influenced by climatic factors, especially temperature and precipitation (via soil moisture) (Lloyd & Taylor, 1994; Davidson *et al*., 2002), and so future increases in global temperature could have a large impact on the global C budget by increasing soil carbon dioxide (CO_2) efflux (Jenkinson *et al*., 1991).

This has led to a number of studies trying to quantify CO_2 flux from soils (e.g. Raich & Schlesinger, 1992; Goulden *et al*., 1996; Maier *et al*., 2011) and the drivers behind the flux (e.g. Wan *et al*., 2007). This is challenging due to bulk soil respiration being the sum of three main processes: microbial decomposition of aboveground litter, microbial decomposition of soil organic matter (SOM) (together heterotrophic respiration) and root respiration (autotrophic respiration) and the associated microbial respiration from rhizodeposition (Cheng, 1996; Lou & Zhou, 2006). Being able to quantify the relative contribution of each source to total respiration is important for calculating vegetation C budgets (since root respiration is independent of soil C pools) and microbial respiration of rhizodeposits and decomposition of SOM can influence the amount of C ultimately stored in soils. However, trying to partition these sources is challenging and currently there is no perfect method to accomplish this, mostly due to the impracticality of separating root respiration from the associated microbial respiration of rhizodeposits. The three main approaches used to partition soil

respiration are experimental manipulations of various components, isotope tracing and inference methods (Hanson *et al*., 2000; Kuzyakov, 2011). One of the most common approaches that has been used since the 1990s includes the removal of root input into soil *via* trenching to estimate root respiration separately (Bowden *et al*., 1993; Boone *et al*., 1998). This technique has since been combined with litter removal to estimate components of soil respiration (litter, roots and SOM) (Rey *et al*., 2002; Sulzman *et al*., 2005). More recently, the use of stable isotopes (Crow *et al*., 2006; Millard *et al*., 2008) has been employed to partition soil respiration through isotopic signal in respired CO_2. The latter has advantages over the former (trenching) due to minimal disturbance of the soil and limiting changes in soil conditions as a consequence of root removal, and increased decomposition through severed roots. However, isotope approaches require that different source materials have different isotopic signatures and that there is no significant fractionation during the processes resulting in soil respiration (Hanson *et al*., 2000). Isotope approaches are also significantly more expensive than physical input manipulation methods, such as trenching.

Different approaches have resulted in high variability of estimates, for example root respiration has been suggested to contribute between 10% and 90% to total soil respiration (Hanson *et al*., 2000). The range of values for contribution of constituent components is also likely to be caused by the inherent differences between ecosystems and the difference in response of constituent parts of soil respiration to changes in climatic variables. For example, Boone *et al*., (1998) suggested that root respiration and microbial respiration have different Q_{10} values and Bhupinderpal-Singh *et al*., (2003) showed that microbial respiration responded to a decline in temperature and root respiration did not. The effects of drought (Borken *et al*., 2006) and C flow in

plants have also been shown to affect root and microbial respiration differently (Högberg *et al*., 2001; Tang & Baldocchi, 2005).

Even with these such limitations in the methods, it is important that we understand the relative contribution of each component to soil respiration in order to assess how this can be affected by future climate (Baggs, 2006). One relatively new platform for such studies is under bioenergy crops used in electricity and heat production, and more recently as a transport fuel. These crops contribute to reducing greenhouse gas (GHG) emissions and remove C from the atmosphere through sequestration in soils. Second generation bioenergy crops (crops for energy production only), have seen a lot of interest over the past decade and are increasingly important politically, becoming an integral component of global legislation (UK Climate Change Act of 2008; UK Renewable Energy Strategy of 2009; European Renewable Energy Directive of 2009; Conservation and Energy Act of 2008, USA). In the UK, one of the most promising bioenergy crops is *Miscanthus x giganteus* (hereafter *Miscanthus*), which is a perennial, C_4 grass, native to Asia and Africa (Lewandowski *et al*., 2000). Given the potential of this crop to provide a low C energy source, it is surprising that there are very few published studies regarding soil respiration from this crop in Europe and UK (Drewer *et al*., 2011; Gauder *et al*., 2011). While there is little information on the contribution of heterotrophic and autotrophic components of this flux in *Miscanthus x giganteus,* there are estimates from *Miscanthus sinensis* (Yazaki *et al*., 2004; Toma *et al*, 2011).

The aim of this study was to partition soil respiration and to establish the relative contribution of autotrophic respiration (defined here as root and rhizome plus associated rhizosphere organisms) and heterotrophic respiration to bulk soil respiration in a *Miscanthus* plantation in Lincolnshire, UK. We used a litter and root

(trenching) manipulation experiment, coupled with measurements of $^{13}CO_2$ to help identify the sources of soil respiration over a 22-month period, covering two growing seasons.

5.3 Materials and Method

5.3.1 Study Site

The field experiment was conducted in a *Miscanthus* plantation in Lincolnshire, UK. The soil type is a clay loam, with approximately 25, 29 and 49%, clay, silt and sand respectively in the top 20 cm of soil. The soil had a mean total C and N content of 1.53 and 0.25% respectively with a soil pH ranging from 6.8 and 7.3. The bulk density of the soil was 1.4 g cm^{-3} and the annual mean water-filled pore space was 82% and 69% for 2009 and 2010 respectively. The site had a mean annual precipitation of 605 mm (30-year average 1980-2010) and the mean annual temperature was 9.9 °C (30-year average).

Please refer to Chapter 3, section 3.3.1, for crop details.

5.3.2 Experimental Manipulations

Five random sampling blocks were established in the *Miscanthus* field. At each of these blocks, five plots (2 m diameter) were installed each with a different treatment; Control (CON), Double Litter (DL), No Litter input (NL), No Root input (NR) and No Root or Litter input (NRL) (Table 5.1). Litter additions were manipulated in CON, DL and NR plots by placing five litter traps (2x2 m) at each sampling block and litter was collected from the traps at each monthly visit during times of litterfall (September to spring harvest). At each monthly visit, the litter collected from each block was combined and weighed. The total weight was then divided by 25 giving the amount of litter for CON and NR plots and this was doubled for the DL plots. The remaining litter was air dried (30°C) and weighed to calculate the biomass inputs to each experimental plot. On the NL and NRL plots, litter was excluded from the plots using

a 20 mm^2 mesh screen and plots were cleared on a monthly basis to ensure these plots were kept clear of litter. Roots were excluded from NR and NRL plots by trenching to a depth of 70 cm, inserting a thick polythene sheet to prevent root in-growth and then back-filled. Due to the compacted nature of the clay soil this depth was sufficient to exclude *Miscanthus* roots. Unwanted new vegetation, mostly mosses, were removed from experimental plots during each monthly field visit.

Table 5.1 – Treatment methods for manipulation plots.

Treatment	Treatment Code	Method
Control	CON	The litter addition was manipulated using five litter traps placed at each block. At each monthly visit, the litter collected from each block was combined and weighted. The total weight was then divided by 25 giving the amount of litter for CON plots.
Double Litter	DL	Plots received double the amount of CON litter.
No Litter	NL	Aboveground litter was excluded from plots using a $20mm^2$ mesh that was cleared on a monthly basis.
No Roots	NR	Roots were excluded from plots by installing an impenetrable barrier after trenching to a depth of 70 cm. Plots received the same amount of litter as CON plots.
No Inputs	NRL	Aboveground litter was excluded as in NL and roots were excluded as in NR.

The trenched plots were installed over 2 months from November 2008 to early January 2009 and measurements commenced in February 2009. Measurements of soil-atmosphere gas fluxes (CO_2, CH_4 and N_2O), gas samples for $^{13}CO_2$ analysis, total C and N stock including $\delta^{13}C$ (0-20 cm depth), litter decomposition and the associated climatic measures of soil and air temperature and soil moisture, were taken on a monthly basis until November 2010.

5.3.3. Soil-atmosphere Gas Fluxes

Gas fluxes (CO_2, CH_4, N_2O and $^{13}CO_2$) were measured using the static chamber method described by Livingston and Hutchinson, (1995) but was adapted to include the use of a fan and pressure 'vent'. The chambers were made from PVC (40 cm diameter and 20 cm height) and were inserted approximately 3 cm into the soil surface (exact volumes noted). All chambers remained in the soil for the duration of the study except for at times of harvest.

At times of sampling, chambers were closed with a reflective aluminium lid, which had a rubber seal around the edge to prevent leakage. Chambers were enclosed for 30 minutes with two 10 ml (one for CO_2 and CH_4 and the other for N_2O) and one 20 ml ($^{13}CO_2$ analysis) sample taken every 10 minutes. At the time of sampling, gas samples were transferred from the chamber headspace into a 3 ml gas-tight exetainer for CO_2, CH_4 and N_2O and 12 ml exetainer for $^{13}CO_2$ (Labco Ltd, UK) via a needle and syringe inserted into the self-sealing septa in the chamber lid. All measurements were taken between 10:15 and 13:15 on the day of sampling. Gas samples of CO_2 and CH_4 were analysed on a Perkin Elmer Autosystem Gas chromatograph (GC) fitted with a flame ionisation detector (FID) and gas samples of N_2O were analysed a Perkin Elmer Autosystem XL GC fitted with an electron capture detector (ECD). All results were calibrated against certified gas standards comprising of 496 ppm CO_2, 1.06 ppm CH_4 and 1.07 ppm N_2O in air (BOC, UK). Gas fluxes were calculated from the change in chamber concentration, field air temperature and chamber volume and area measurements using the method in Holland *et al*., (1999). Analysis of $^{13}CO_2$ is described later.

Climatic conditions were noted at each sampling visit at the time of gas sampling. This included measurements of air and soil temperature using a Tiny Tag temperature

logger with integral stab probe (Gemini Data Loggers, UK) and volumetric soil moisture (0-6 cm depth) measurement using a ML2x Theta Probe and Meter HH2 (Delta T Devices, UK). This was determined by taking the mean of three measurements taken close to each chamber during gas measurements.

5.3.4. Soil Sampling and Analysis

Fresh soil samples (0-20 cm, 2 cm diameter) were taken from all plots using an auger within each plot on a three monthly basis. The samples were split into 0-10 cm and 10-20 cm sections, from which a subsample was taken, ground and analysed for total C and N content using a TruSpec CN analyser, (LECO, UK).

5.3.5 Isotopic Analysis

The $\delta^{13}C$ of CO_2 was determined by trace gas – isotope ratio mass spectrometry (TG-IRMS). That is to say, an Isoprime isotope ratio mass spectrometer was coupled to an Isoprime Ltd trace gas pre-concentration unit (Isoprime Ltd, Manchester, UK). One hundred microlitres of gas were removed from each vial and injected using a gas-tight syringe into the TG-IRMS. The sample was then diverted through a trap filled with magnesium perchlorate to remove water, after which the CO_2 was cryogenically concentrated in glass lined cryofocussing traps immersed in liquid nitrogen. Prior to entering the IRMS, the CO_2 was separated from other non-condensable gases on a 30 m gas chromatography capillary column filled with Poraplot Q. Reference standards of known isotopic composition were included after every fifteenth sample during analysis. Internal precision was better than ± 0.2 ‰ at 1 σ for $\delta^{13}C$ for the reference standards.

5. Partitioning soil respiration in *Miscanthus*

The $\delta^{13}C$ values of soil samples was determined on an automated Eurovector elemental analyser coupled to an Isoprime Isotope Ratio Mass-Spectrometer (Isoprime Ltd, Manchester, UK). An in-house soil standard was analysed after every twelfth sample resulting in an analytical precision of 0.29‰.

Isotopic data are reported using the delta notation with $^{13}C/^{12}C$ variations relative to the international standard Vienna Pee Dee Belemnite (V-PDB):

$$\delta^{13}C\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000 \qquad [1]$$

where R is the ratio (absolute) of the isotopes (heavy to light) being compared and differences in the ratio between a standard and sample are reported in parts per thousand or per mil (‰).

The keeling-plot approach (Keeling, 1958) was used to estimate $\delta^{13}C$ value from respired CO_2 for each plot for each treatment. Keeling's method showed that the integrated $^{13}CO_2$ signal produced by all components of soil respiration could be determined as the intercept of a regression of $\delta^{13}C$ *versus* the inverse of CO_2 (ppm), where both values were collected at the same time point during chamber enclosure.

5.3.6 Litter Decomposition and Analysis

Litter decomposition was measured using the litterbag technique (Olson JS, 1963) so as to determine any additive effects of extra litter (i.e. CON vs DL) on litter decomposition rates. Litterbags were made from a 1 mm nylon mesh (PlastOk Ltd., UK), measuring 20 by 10 cm and were filled with approximately 5 g of air dried litter, first cut into 15 cm lengths, that was collected from the field in October 2008. Litterbags were pinned to the soil in the litter layer of CON, DL and NR plots in

November 2009 and were recovered from the field after 1, 4, 8 and 12 months (60 bags in total). A set of 'travel bags' was also constructed to determine the mass loss of litter due to transport to the field (St. John *et al*., 2011). On removal from the field, external debris was removed and then the litter was carefully cleaned to remove all remaining debris (mostly soil). Mass loss was determined by drying litter at 80°C for 24 hrs and was expressed as a function of initial mass. Subsamples of litter were taken for total C and N content, which were freeze-milled and then analysed using a LECO TruSpec CN analyser. Subsamples were taken from initial litter and 12 months litter bags and analysed for fibre, cellulose and lignin using acid-detergent fibre sulphuric acid procedure as described in Rowland and Roberts, (1994).

5.3.7 Partitioning Soil Respiration

Total soil respiration as measured from CON plots (R_T) is the result of three main process; above-ground litter decomposition (R_L), rhizosphere respiration (R_R) (root, rhizome and associated rhizosphere organisms) and respiration resulting from the decomposition of SOM and fine root turnover (R_{SOM}). The relative contribution of these processes to R_T is estimated by comparing respiration from various treatments (Hanson *et al*., 2000). The contribution from R_L was estimated by subtracting NL respiration from CON respiration, R_R contribution is estimated by subtracting NR from CON and the R_{SOM} contribution is from the NRL plots.

5.3.8 Modelling Annual Soil Respiration

Measurements of soil respiration, temperature and soil moisture from each monthly sampling were used along with continuous measurements of air temperature (Platinum

Resistance Thermometer, Didcot Instruments Ltd., UK) and soil moisture (20 cm depth) taken from an automatic meteorological station adjacent to the *Miscanthus* plantation, to model hourly soil respiration for 2009 and 2010. Measured air temperature, soil temperature and soil moisture were used to create individual relationships for each treatment in 2009 and 2010 to generate treatment specific continuous soil temperature and soil moisture measurements. There was a strong correlation between measured air and soil temperature (R^2=0.98, 2009; R^2=0.97, 2010) so these relationships were used to model continuous soil temperature for each year, respectively. Continuous soil moisture measurements were generally higher than measured soil moisture so these were adjusted using the relationship between measured soil temperature and measured soil moisture (R^2=0.71, 2009; R^2=0.72, 2010). The monthly measurements of soil temperature and soil moisture were used to generate multiple linear regression models for each treatment. The model used to best explain soil respiration for each treatment was:

$$\text{Transformed } (R_T) = \text{soil temperature} + \text{soil moisture} \quad [2]$$

Continuous soil temperature and moisture measurements were used in equation [2] to generate hourly soil respiration for each treatment. Soil respiration from CON and DL plots was log-transformed and soil respiration from NL, NR and NRL plots was square-root transformed to normalise data.

5.3.9 Statistical Analysis

All statistical analyses were carried out using R, version 2.14.0. Where data was normally distributed or could be easily transformed by log or square root

transformation, linear mixed effects (LME) models were used to assess for differences in gas fluxes, temperature, soil moisture and C:N content of litter bags over the study period. Where data could not be easily transformed to be normally distributed (mass remaining in litter decomposition), analysis was performed using a generalised linear mixed effects (GLME) model allowing for non-normal data. Here we used gamma distribution. In both models (GLME and LME) random effects were included to account for differing levels of variation within sampling location to between sampling location and within time (days) to between time. Fixed effects of interaction between treatment and time were tested for. Where individual months were assessed for difference between treatments a one-way ANOVA was used. Where significant differences were found ($p<0.05$), post-hoc comparisons were made used made using Fisher's LSD test.

5.4 Results

5.4.1 Soil Temperature and Soil Moisture

Soil temperature varied markedly with season, with maximum temperatures reached in July and minimum temperature in February of both years (Figure 5.1a). There was no significant difference between treatments ($p>0.05$) in yearly mean soil temperature. However on a monthly basis, Dec-09, Jan-10 and Feb-10 NL and NRL litter treatments had significantly lower soil temperatures ($p<0.05$) than the other treatments and Jun-10 and Jul-10 NL and NRL treatment had significantly ($p<0.05$) higher soil temperatures than the other treatments.

Soil moisture was not significantly different between treatments on an annual basis ($p=0.094$). Soil moisture varied greatly with season and significantly between years 2009 and 2010 ($p=0.008$), with 2009 being overall drier than 2010 (Figure 5.1b). In 2009 soil moisture was more variable than in 2010 and responded quickly to heavy precipitation (Figure 5.1c) and high temperatures.

5.4.2 Soil Respiration

Soil respiration from all treatments followed a seasonal pattern with higher fluxes over the summer months (July to September) and lower fluxes over the winter months (December to February) (Figure 5.2a). There was a significant difference ($p=0.003$) in soil respiration between 2009 and 2010, suggesting lower total soil respiration in all treatments in 2010 than 2009 and is supported by Figure 5.3a. There was a lower peak respiration from CON plots of 53.5 mg CO_2-C m^{-2} h^{-1} in August compared to two peaks of respiration in 2009 of 159.2 mg CO_2-C m^{-2} h^{-1} and 217.9 mg CO_2-C m^{-2} h^{-1} in July and September respectively.

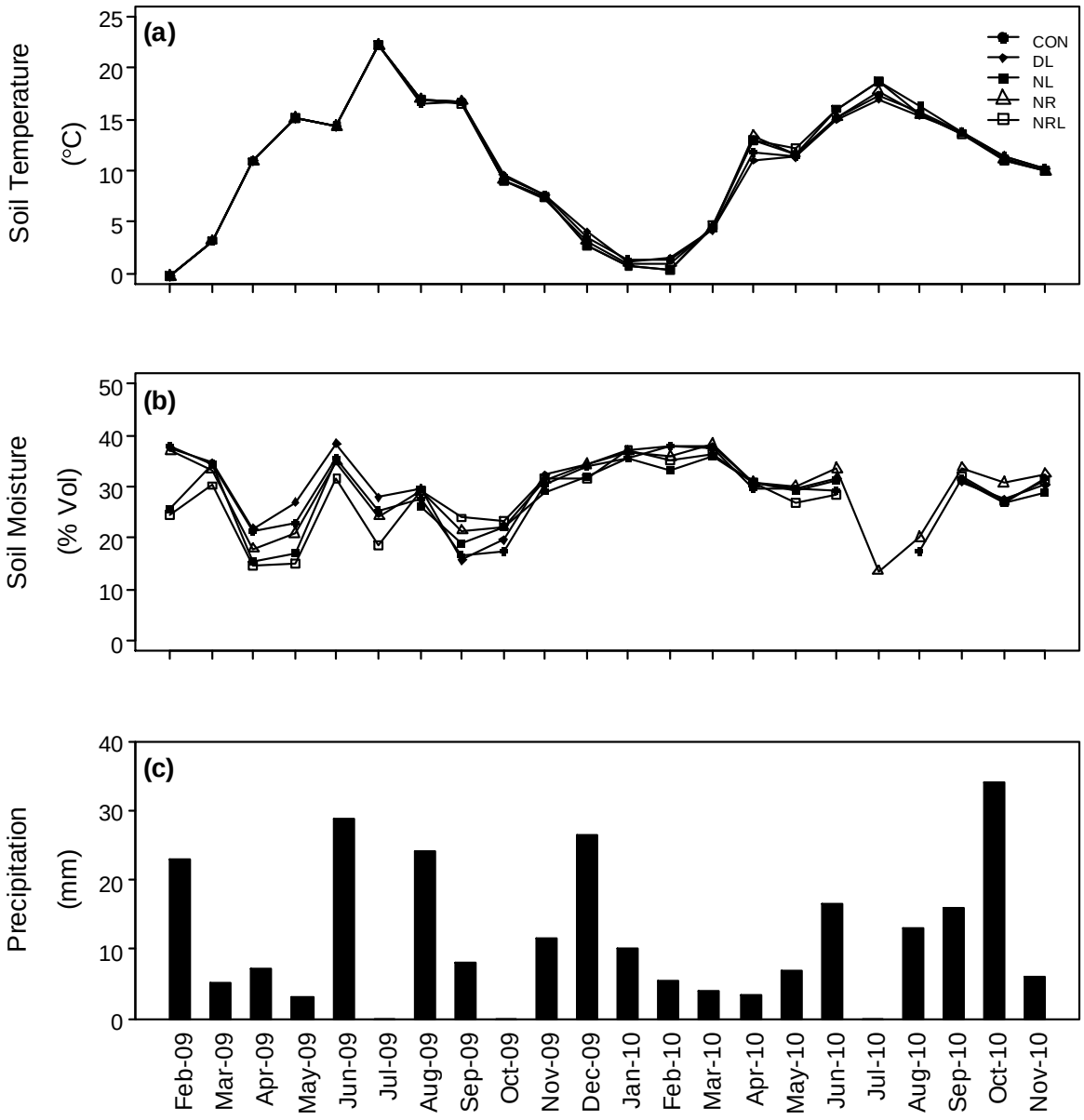

Figure 5.1 – Mean monthly values (n=5) of **a)** Soil temperature (0-5 cm), **b)** Soil moisture (0-6 cm) and **c)** Precipitation as sum of precipitation 7 days before sampling (mm). Closed circle is Control (CON), closed diamond is Double Litter (DL), closed square is No Litter (NL), open triangle is No Roots (NR) and open square is No Roots or Litter (NRL).

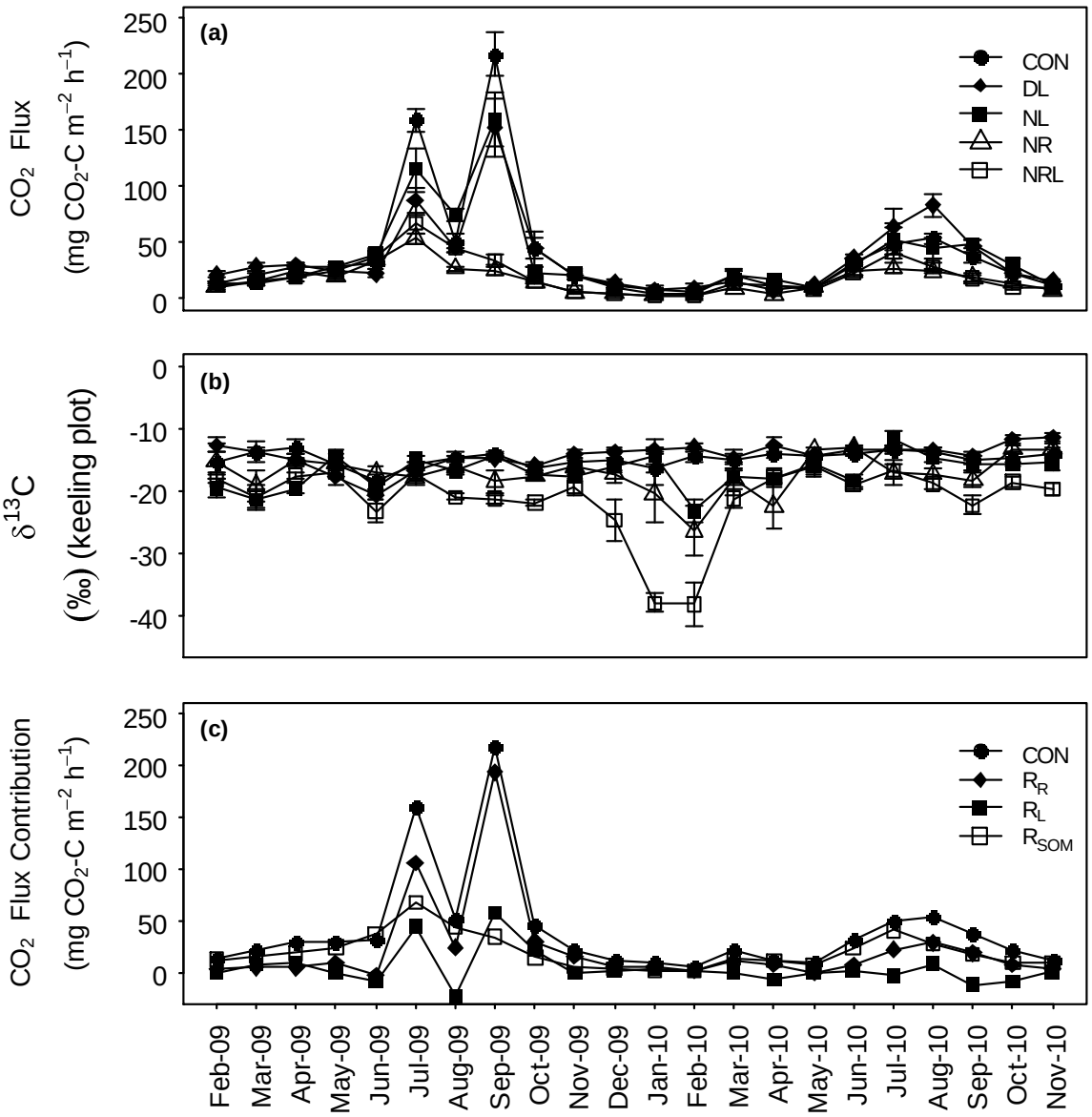

Figure 5.2 – Mean monthly (n=5) values of **a)** soil respiration as measured from all treatments from February 2009 to November 2010, **b)** the $\delta^{13}C$ value derived from Keeling plots and **c)** the seasonal variation in soil respiration components. For **a)** and **b)** closed circles is Control (CON), closed diamond is Double Litter (DL), closed square is No Litter (NL), open triangle is No Roots (NR) and open square is No Roots or Litter (NRL), with bars representing standard error values. For **c)** closed is CON, R_R is rhizosphere contribution, R_L is litter contribution and R_{SOM} is the contribution from soil organic matter (SOM), all values are calculated from measured respiration.

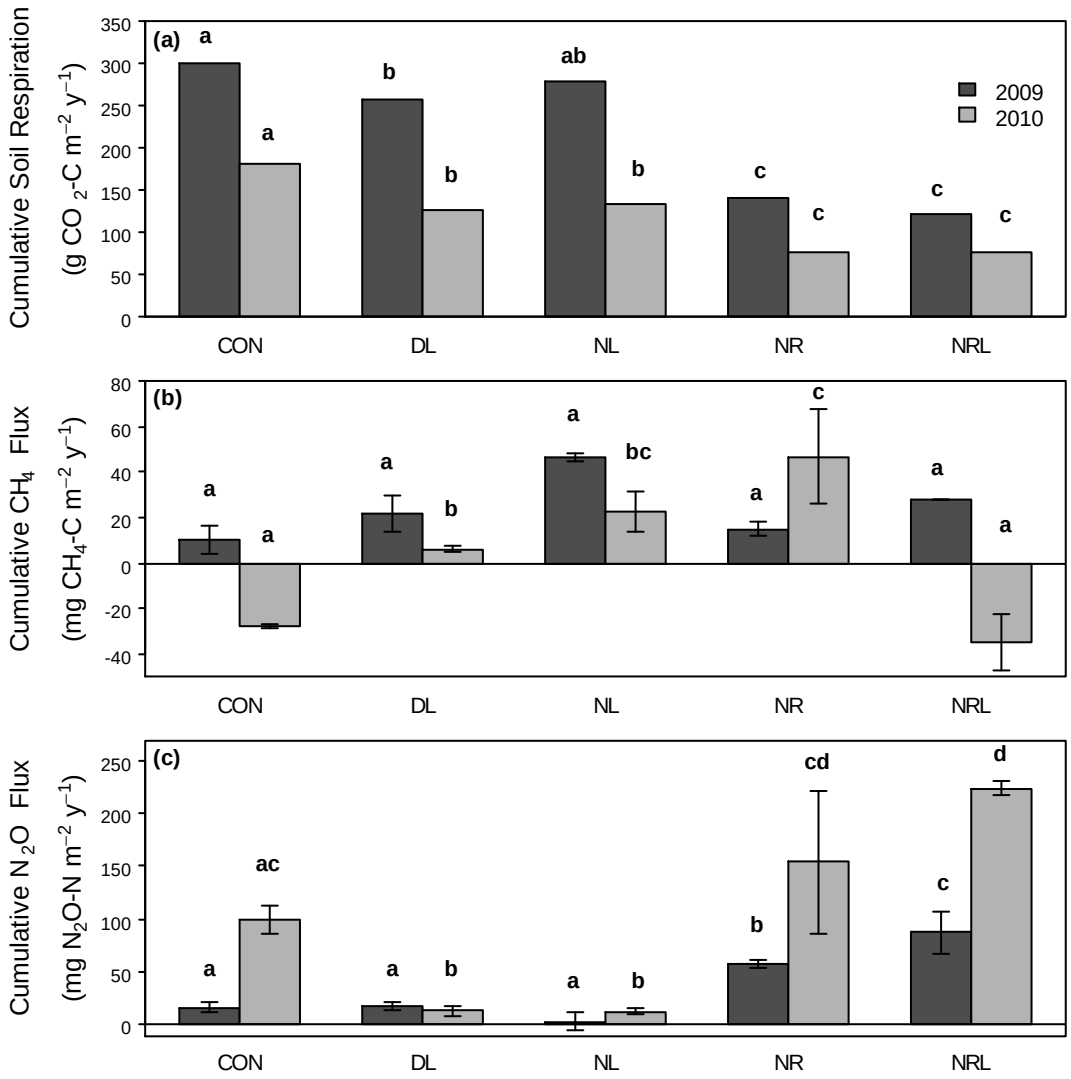

Figure 5.3 – Cumulative soil-to-atmosphere fluxes for 2009 (dark grey bars) and 2010 (light grey bars) of **a)** Soil respiration, **b)** Methane (CH_4) and **c)** Nitrous oxide (N_2O). Values for soil respiration are cumulative modelled fluxes. Values for CH_4 and N_2O are extrapolated from the mean cumulative sums from each plot and bars represent standard error values. Significant differences (ANOVA and pair-wise post-hoc comparisons, $p<0.05$, at 1, 23 *df)* between treatments in each year are indicated by different letters.

5.4.2 Soil Respiration

All treatments showed a significant correlation with temperature (air and soil) and all but NL showed a significant correlation with soil moisture, although the strength of the relationship varied (Table 5.2). The strongest correlation with both soil and air temperature was with NRL (r=0.77 and r=0.78, respectively), followed by NL, CON, NR and DL. Soil moisture followed a different pattern with the strongest correlation with CON (r=-0.61), then NR, NRL, DL then NL.

Table 5.2– Pearson correlation coefficient and significance between each treatment respiration and air temperature, soil temperature and soil moisture (0-6 cm depth).

	Air Temperature		Soil temperature		Soil moisture	
	r	p	r	p	r	P
CON†	0.70	<0.001	0.69	<0.001	-0.61	<0.001
DL†	0.55	<0.001	0.58	<0.001	-0.41	<0.001
NL‡	0.73	<0.001	0.71	<0.001	-0.21	0.10
NR‡	0.65	<0.001	0.69	<0.001	-0.49	<0.001
NRL‡	0.78	<0.001	0.77	<0.001	-0.43	<0.001

† Respiration log transformed
‡ Respiration square root transformed

There was a significant difference in soil respiration rates between treatments ($p<0.0001$) over the study period, with both no root treatments (NR and NRL) showing lower respiration rates than CON plots. This is shown in Figure 5.3a, where there are significantly lower ($p>0.05$ ANOVA) cumulative CO_2 emissions for both NR and NRL treatments compared to the other treatments. When looking at the data on a monthly basis there were several months, mostly from after the harvest to the first peaks in temperature (Jul-09 and Aug-10), where fluxes from NR and NRL plots were not statistically different from CON fluxes (Figure 5.2a) ($p>0.05$, one-way ANOVA).

Both DL and NL plots showed no significant difference (p=0.369, p=0.152 respectively) in mean respiration rates compared to CON plots over the whole study period. However, the cumulative fluxes in Figure 5.3a for DL and NL were lower than that of CON in both years and DL fluxes were significantly lower ($p<0.05$) in 2009 and both DL and NL fluxes were significantly lower ($p<0.05$) in 2010.

5.4.3 CH_4 and N_2O Fluxes

Although CH_4 fluxes and N_2O fluxes were not the main focus of the study, they were measured from each of the plots and the cumulative fluxes are shown in Figure 5.3bc. For CH_4, due to the low fluxes and high variability of the data no significant treatments effects were found (p=0.707) in 2009 (Figure 5.3). In 2010 there was a significant treatment effect (Figure 5.3), with CON and NRL plots showing negative fluxes compared to the other treatments. N_2O fluxes were generally higher in 2010 than 2009 but no significant difference was found between years (p=0.127). There was a significant difference between treatments ($p<0.001$) over the whole study period, with NR and NRL treatments showing higher N_2O fluxes and NRL showing significantly higher fluxes (p=0.02). NR and NRL plots showed significant correlations with air and soil temperature (Pearson, r=0.33, $p<0.01$), but the other treatments showed no significant correlation with any environmental parameters ($p>0.05$).

5.4.4 Contribution to Total Soil Respiration

The contribution to total respiration from R_L, R_R and R_{SOM} was determined from modelled soil respiration and is shown for both years in Figure 5.4. The majority of

total flux came from the R_R and R_{SOM} in both years. The litter contribution varied between years, with very little (<10%) contribution in 2009 (Spring, Summer, Autumn) compared to 20% contribution in 2010 (Spring, Summer, Autumn). The contribution from litter increased in the winter for both years, seeing an increase to 25% in 2009 and 34% in 2010. Due to the increased contribution from litter in 2010 the contribution to total flux from the R_R and R_{SOM} was less than in 2009. There was a seasonal pattern to all constituent parts forming total soil respiration, but most of all by R_R (Figure 5.2c). R_{SOM} showed peaked in both years in July and then showed a decline, but this also corresponded with depletion in $\delta^{13}CO_2$ values (Figure 5.2c) which may indicate a switch in C resource. R_L showed the least seasonal pattern with soil temperature but tended to show increases in contribution when soil moistures were higher, around 30%.

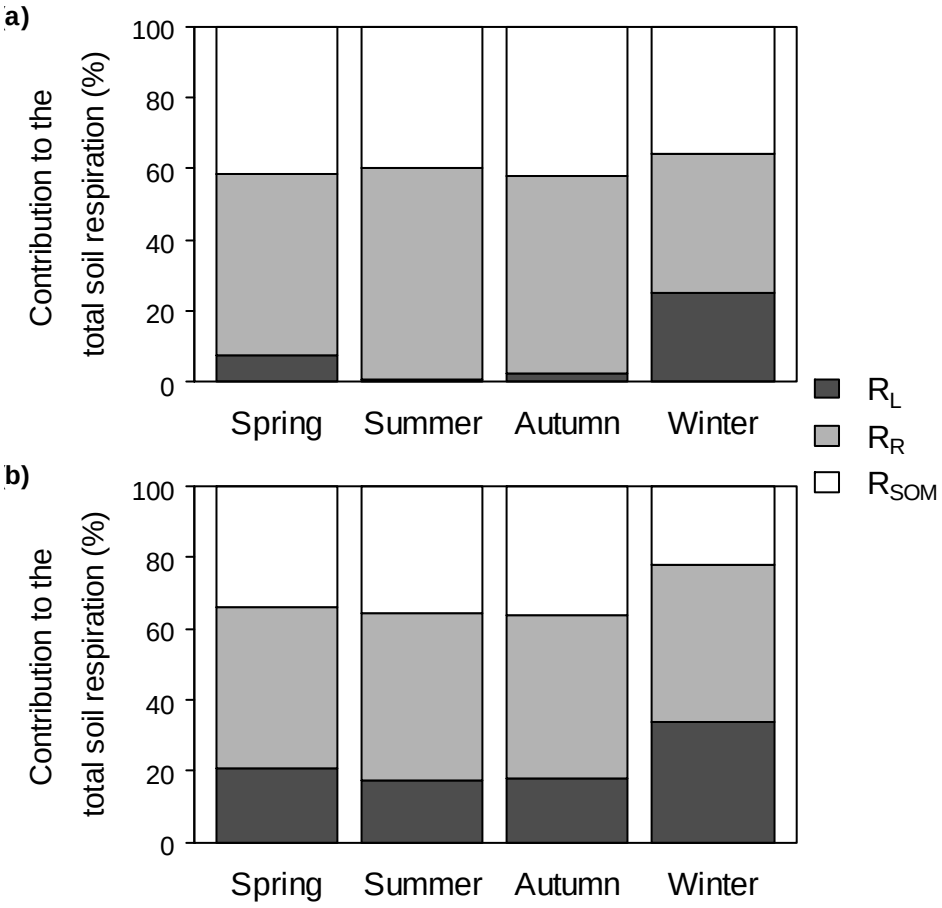

Figure 5.4 – The contribution of aboveground litter decomposition, root respiration and SOM to total soil respiration for **a)** 2009 and **b)** 2010 in four seasons, spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). Values used were from the modelled CO_2 fluxes.

5.4.5 δ $^{13}CO_2$

Monthly changes in $\delta^{13}C$ from CO_2, as derived from keeling plots, for each treatment are shown in Figure 5.2b. The isotopic signature from CON plots showed no seasonal pattern and excluding June 2009, when the signature dips to -18.6 ‰, the isotopic signature only varied by 3.4 ‰ and had an average over the study period of -14.8 ‰ (±0.21 S.E.). There was no significant difference ($p>0.05$) between the mean isotopic signature of DL and CON, although there was a more pronounced depletion of $\delta^{13}C$ in June 2009, when the isotopic value was -20.7 ‰. This suggests that both these fluxes are derived from more enriched sources of ^{13}C derived from *Miscanthus*. The mean $\delta^{13}C$ value from NL plots was -17.0 ‰ and there was a significant difference ($p=0.003$) between the isotopic signature of NL and CON plots suggesting that NL plots are more depleted in $\delta^{13}C$. There was a large variation of 11.6 ‰ between the minimum and maximum values for NL plots, with the most depleted signatures observed around the time of harvest in March 2009 (-21.5 ‰) and February 2010 (-23.3 ‰). This suggests that at times of harvest the flux from NL plots draws on older C_3 source of C. The mean $\delta^{13}C$ value from NR plots was -17.3 ‰ and again there was a large variation in signature with the most depleted values coming in Feb-10 (-26.5 ‰) and Apr-10 (-22.5 ‰). There was a significant difference between the $\delta^{13}C$ of CON and NR plots ($p=0.038$). NRL plots showed a highly depleted signal in Jan-10 and Feb-10 and generally had a more depleted signal than CON plots in all months of the study, with a mean of -21.4‰.

5.4.6 Soil C and δ ^{13}C

Soil sample analysis of total C content in cores taken on a three-monthly basis showed no significant difference ($p>0.05$) in total C content between treatments at the end of

the study (Figure 5.5a). There were also no significant difference ($p>0.05$) found in C content at different depths for each treatment. However, there were significant differences in the $\delta^{13}C$ between treatments ($p<0.001$) at 0-10 cm, with significant differences found between CON value and NL and NRL plots (Figure 5b). This suggests that a lack of litter input does result in soil beginning more depleted in $\delta^{13}C$. No significant difference ($p>0.05$) was found between treatments at 10-20 cm. A significant difference ($p<0.05$) was found between soil depths for each treatment, and in each case 10-20 cm depth of soil was more depleted in $\delta^{13}C$.

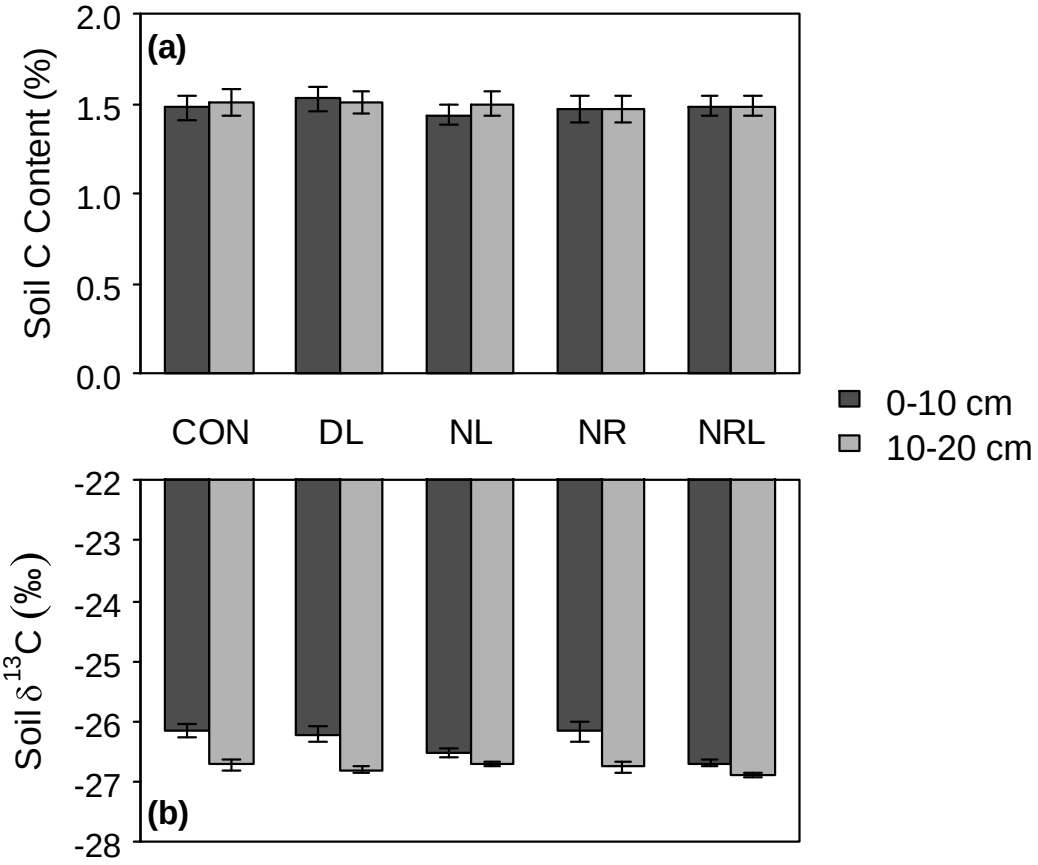

Figure 5.5 – Mean values over the whole study of **a)** total soil C content (%) and **b)** $\delta^{13}C$ values (‰) for two depths of 0-10 cm and 10-20 cm for each treatment. Error bars represent standard error values.

5.4.7 Litter Input and Decomposition

Litter input to SL, NR and DL plots was estimated from the dry weight (dw) of the remaining litter after litter was added to the plots (Table 5.3). The litterfall period (LFP) for was from September to the spring harvest in both years of this study. The litter input into the plots in the 2009-2010 LFP was 2.9 t dw ha^{-1}, which was only slightly higher than litter input over the LFP for 2010-2011. This was reflected in the C and N inputs from litter in both LFP.

Table 5.3 – The litter input, C and N input from litter, for each litterfall period (LFP, September to harvest), for CON, NR and DL plots.

	SL and NR	DL
2009-2010		
Litter input (t dw ha^{-1} LFP^{-1}) ‡	2.86	5.71
C input (t C ha^{-1} LFP^{-1}) ‡†	1.20	2.40
N input (t N ha^{-1} LFP^{-1}) ‡¶	0.03	0.07
2010-2011		
Litter input (t dw ha^{-1} LFP^{-1}) ‡	2.37	4.75
C input (t C ha^{-1} LFP^{-1}) ‡†	1.00	1.99
N input (t N ha^{-1} LFP^{-1}) ‡¶	0.03	0.06

‡LFP is Litter Fall Period. In each period of litter fall was from September to harvest in spring
† litter C content was 42%
¶ litter N content was 1.2%

Litter decomposition showed no significant difference in mass loss between treatments ($p=0.400$). All treatments showed similar mass loss dynamics although there was less mass loss from NR litterbags in months 4 and 8 compared to bags from CON and DL treatments (Figure 5.6a). There was a significant effect of time ($p<0.001$) but no interaction effect was found between treatment and time. Similar

results were found for the C:N ratio (Figure 5.6b), with no significant treatment effect ($p=0.123$) but a significant time effect ($p=0.003$) suggesting a decrease in C:N ratio with time.

Although no significant difference was found in mass loss between treatments, there was a difference in the nutrient release and the loss of fibre, cellulose and lignin between treatments in 12-month litter bags (Table 5.4). There was no significant N release between treatments after 12 months ($p>0.05$), but C release showed a difference between CON and NR plots suggesting a higher release of C from NR plots. This is likely due to the high loss of lignin from NR litterbags compared to the other treatments ($p<0.05$), although there was less cellulose loss than the other treatments. The only significant difference between CON and DL litterbags was in fibre loss ($p<0.05$), with CON litterbags having approximately 10% higher loss (Table 5.4).

Table 5.4 - Nutrient release and loss of three chemical components from litter bags after 12 months in the field for each treatment (Control (CON), Double Litter (DL) and No Roots (NR)). Values are means ± standard error, with the number of samples (n) shown in brackets. No significance difference was found between treatments for each parameter when letters are the same ($p>0.05$).

	N Release (%)	C Release (%)	Loss Fibre (%)	Loss Cellulose (%)	Loss Lignin (%)
CON	53.5 ± 4.0 [a] (n=4)	60.0 ± 2.6 [b] (n=4)	52.4 ± 1.2 [a] (n=5)	62.7 ± 1.8 [a] (n=5)	50.7 ± 2.2 [b] (n=4)
DL	52.7 ± 2.7 [a] (n=4)	64.0 ± 2.9 [ab] (n=4)	42.4 ± 2.1 [b] (n=4)	53.8 ± 3.7 [a] (n=4)	31.1 ± 2.5 [b] (n=4)
NR	51.5 ± 4.0 [a] (n=5)	69.1 ± 2.5 [a] (n=4)	48.2 ± 0.7 [ab] (n=4)	35.9 ± 1.7 [b] (n=5)	81.5 ± 1.7 [a] (n=5)

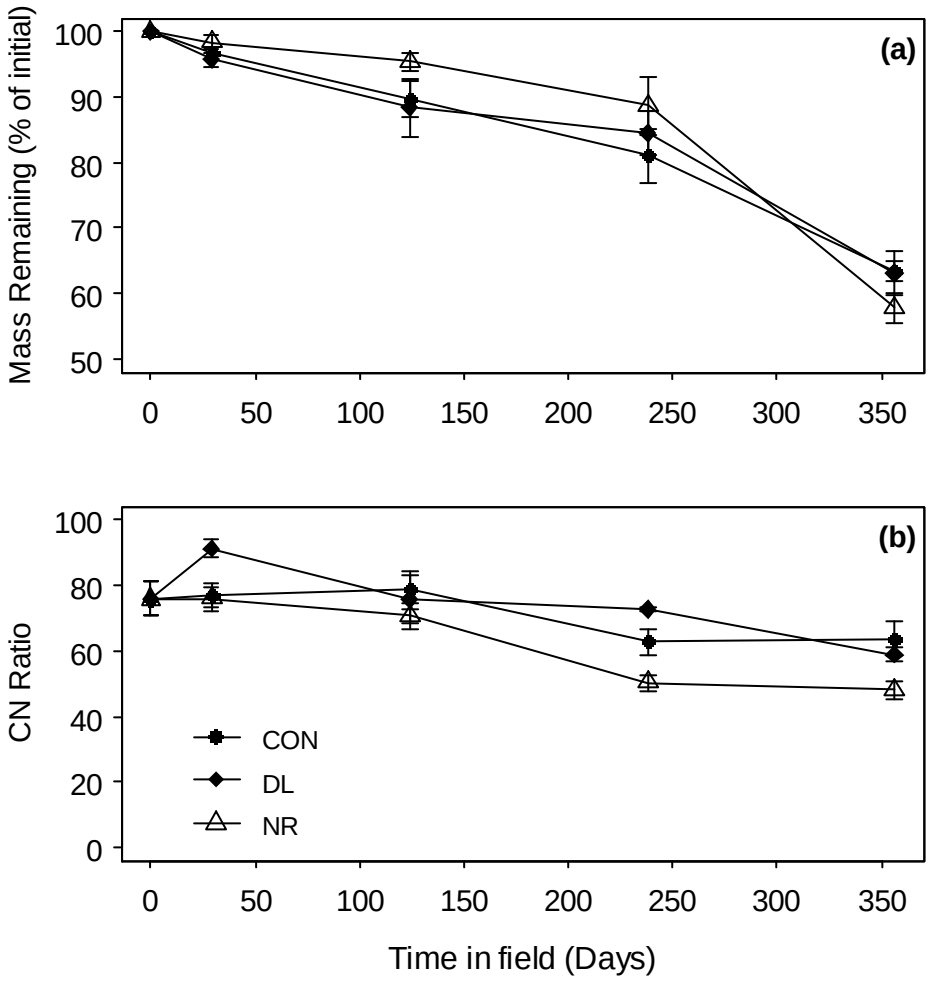

Figure 5.6 – Mean monthly values (n=5) of **a)** mass remaining (% of initial) and **b)** C:N ratio for litter bags place in Control (CON) (closed circle), Double Litter (DL) (closed diamond) and No Root (NR) (open triangle) plots. Error bar represent standard error values.

5.5 Discussion

To our knowledge, this is the first experiment of this type in *Miscanthus* and is important for improving our understanding of the components of soil respiration, since this crop is of such potential importance in terms of providing a low C energy source.

5.5.1 Effects of Temperature and Moisture on GHG Fluxes

The seasonal pattern of soil respiration generally matched that of soil temperature and significant correlations were found with all treatments (Table 5.2). The dependence of soil respiration on temperature is well documented and there are many studies that have reported this relationship (Lloyd & Taylor, 1994; Gu *et al*., 2008; Drewer *et al*., 2011). CH_4 fluxes were minimal and showed no seasonal trend, which was also found by Gauder *et al*., (2011), and there were no treatment differences in 2009 and no significant correlations with environmental parameters in both years. In 2010, there was a significant treatment difference, but due to the variability of the data and the minimal nature of the flux it is difficult to determine if this is a real effect. N_2O fluxes from CON plots showed no seasonal trend and no correlation with environmental parameters. There was a significant difference between treatments, with NR and NRL showing higher fluxes, which is likely to be due to higher N availability to soil microbes (i.e. not taken up by plant roots).

The effect of soil moisture on respiration is often less clear due to soil temperature often being confounded with soil moisture (Davidson *et al*., 1998). This study showed mixed results between treatments with CON plots having a strong negative relationship with soil moisture (Table 5.2) but NL plots having no significant correlation with soil moisture, suggesting the importance of litter in regulating soil moisture content. Soil moisture often has limiting effects on soil respiration when at

extremes and can inhibit respiration through preventing CO_2 diffusion when too high and can stress microbial communities, reducing total respiration when too low (Bowden *et al*., 1998). Soil moisture content did drop below 20% in the top 10 cm of soil, usually corresponding to high temperatures, which may have limited soil respiration in the summer months (Rey *et al*., 2002). Clifton-Brown and Lewandowski, (2000b) showed that water-limiting conditions resulted in leaf senescence, which could result in lower soil respiration due to lower photosynthetic capacity and C supply to the roots. However, Xu *et al*., (2004) observed that when soil respiration was limited by soil moisture, there was also a rapid increase in soil respiration after precipitation events, which was not observed in this study. However, this may be due to these 'events' being missed due to our campaign based sampling technique.

5.5.2 Contribution to Soil Respiration

5.5.2.1 Litter Contribution

The contribution from litter decomposition varied between years and with season, with a much lower litter contribution in 2009 (7%), the driest year, compared to 2010 (21%). Our estimates from 2010 are similar to other published estimates by Rey *et al*., (2002; 22%), Sulzman *et al*., (2005; 19%) and Li *et al*., (2004; 20%). Rey *et al*., (2002) also reported increased litter contribution in different seasons, seen in both winters (Dec to Feb) in this study, and attributed this to high rainfall when leaves started to fall. In this study, the increase is likely to be due to similar reasons including increased and sustained higher soil moisture during winter, allowing litter that fell from the end of the growing season (September) (Beale & Long, 1997) onwards to be decomposed more readily. The results from the litter decomposition study support this

by showing a higher rate of decomposition after 120 days of being in the field, which would coincide with the higher litter contribution to soil respiration in winter (Figure 5.6).

2009, as the driest year, had a much lower litter contribution in spring, summer and autumn compared to the same seasons in 2010 suggesting soil moisture plays a role in litter decomposition. Cisneros-Dozal *et al*., (2007) found similar results when respiration increased due to litter decomposition when litter was wetted after a period of drought. The lower soil moisture content in these seasons may have been too low in some months for litter decomposition by soil organisms, resulting in a lower litter contribution to total soil respiration. Cisneros-Dozal *et al*., (2006) found that changes in leaf litter moisture were primarily responsible for changes in soil respiration and accounted for 1% of total soil respiration in dry conditions and up to 42% in wet conditions, but this was in a hardwood forest so values are likely to be different in *Miscanthus*. Although litter contributed the least to soil respiration, the contribution of litter C to soil was clear by the significant difference found in $\delta^{13}C$ value soil between littered and non-littered treatments and between the two different depths studied (Figure 5.5).

The contribution of litter decomposition to overall CO_2 flux may be lower than the other contributors due to the 'Gadgil' effect. Gadgil and Gadgil (1971, 1975) found that when mycorrhizal roots were excluded from litter, the rate of litter decomposition increased over a period of 12 months. It is thought that mycorrhizae fungi inhibit saprophytic organisms that decompose litter. It has been shown that Miscanthus roots do form associations with arbuscual mycorrhizae (AM) fungi (An *et al*., 2008) and the results from the decomposition experiment support this idea with a higher rate of decomposition from NR plots in the 12^{th} month of the study (Figure 5.6) compared to

CON and DL plots. This suggests that AM fungi may be playing a role in litter decomposition but more evidence is required.

5.5.2.2 Rhizosphere Contribution

Our estimates of the rhizosphere contribution varied from 44% to 59%, with the highest contribution from summer in 2009, when litter contribution was at its least. This is similar to estimates of root respiration of 22% to 53% measured in *Miscanthus sinensis* from Yazaki *et al*., (2004), with our study showing a slightly higher contribution due to including microbial respiration from rhizodeposits. Toma *et al*., (2010a), found that heterotrophic respiration was one of the dominant components to the C budget they calculated from a *Miscanthus sinensis* grassland, which is similar to this study. However, Toma *et al*., (2010a) calculated that heterotrophic respiration to be in the range of 2.3 to 3.1 t C ha^{-1}, which is higher than the estimate of 1.5 t C ha^{-1} calculated in this study. The main reason for this may be that Toma *et al*., (2010a) were working in a well-established 30-year old grassland compared to our 3 or 4 year old plantation, which would make a large difference to the overall productivity of this *Miscanthus*.

Our estimates are also within the range of 30% to 70% of rhizosphere respiration estimated in maize by Ding *et al*., (2007) and close to maximum estimates of 45% by Rochette *et al*., (1999) from maize. Other estimates from studies investigating rhizosphere respiration in forests ecosystems also return estimates encompassing our own, ranging from low estimates of 23% (Sulzman *et al*., 2005) and 33% (Bowden *et al*., 1993) to higher estimates of 51% (Nakane *et al*., 1996) and 60% (Epron *et al*., 1999). Many of these forest studies suggest that the proportion of root respiration to total soil respiration stays fairly constant when the forest is close to equilibrium,

suggesting that the root contribution in *Miscanthus* may become more consistent when the crop reaches maturity and comes out of the establishment phase as in this study.

We found rhizosphere respiration to be influenced by season (Figure 5.2c), which has also been found in a number of other studies (Epron *et al*., 2001; Högberg *et al*., 2001; Rey *et al*., 2002; Yazaki *et al*., 2004; Ding *et al*., 2007). Rhizosphere respiration was generally low until June, increased over July to September and then decreased from October onwards. This is likely to be closely linked to plant productivity as *Miscanthus* growing season matches this pattern with the growing season starting shortly after the harvest in spring, continues to approximately late July beginning of August, and then no further growth hereafter due to senescence (Beale & Long, 1997). Other studies have also found links between photosynthesis and changes in rhizosphere respiration (Högberg *et al*., 2001; Cisneros-Dozal *et al*., 2006), and is supported by Neukirchen *et al*., (1999) who found an increase in *Miscanthus* root biomass over the growing season, linking this to increased rhizosphere respiration over the growing season. The decline in rhizosphere respiration at the end of the growing season is linked to the beginning of leaf senescence at the end of the growing season and the recalling of nutrients to the rhizome at the end of the growing season, also reducing rhizosphere respiration (Beale & Long, 1997).

Our estimates of rhizosphere respiration are likely to have been affected by decomposition of severed roots during the experiments installation, though this was done during the plant senescent phase and several months had passed before measurements started. The decomposition of roots may have overestimated respiration from NR plots and resulted in an underestimation of rhizosphere respiration (estimated by CON-NR), which has also been seen in other studies (Bhupinderpal-singh *et al*., 2003). However, the exact amount that root decomposition contributed to

respiration in NR plots is difficult to determine and is likely to have been confounded by the effects of season as reflected by our results showing lower rhizosphere respiration in 2010 as a result of lower temperatures. Sulzman *et al*., (2005) suggested that it is possible for rhizosphere respiration to be overestimated by soil organisms switching to alternative sources of C (e.g. SOM), rather than assuming that soil organisms associated with the rhizosphere die soon after trenching. This appears unlikely from our $\delta^{13}CO_2$ results, since there was no major depletion (average 1.1‰ ±1.2 SE) in the $\delta^{13}C$ of NR respiration compared to CON respiration in the first few months of the study although this may be affected by the isotope signal from litterfall into these plots.

5.5.2.3 SOM Contribution

The contribution from the decomposition of SOM ranged from an average of 40% in 2009 to 33% in 2010. This is similar to Bowden *et al*., (1993) who estimated that 30% of total soil respiration was contributed by SOM, but is lower than other estimates from Sulzman *et al*., (2005) and Rey *et al*., (2002), who suggest that SOM contribution was 58 ± 10% and 55% respectively. These latter estimates were both taken from well established forests and our lower estimated could be due to the nature of the crop and its age. It is clear from the isotopic results from respired CO_2 (mean -21.0‰) and soil (mean -26.5‰), that *Miscanthus* has contributed to SOM. For the most part, SOM decomposition was most likely *Miscanthus* derived but there was a clear shift to old (C_3) organic matter in Jan and Feb of 2010 but this did not result in higher fluxes from NRL plots. This could be attributed to a shift in C source by microbes in winter, which is supported by Pelz *et al*., (2005), who suggested that the microbial community used different C sources over the growing season in *Miscanthus*

sinensis. Other studies have also found shifts in C source utilisation over the winter in different ecosystems (Lipson *et al*., 2002; Sacks *et al*., 2005). This source switch is likely to be amplified by the lack of litter and highlights the importance of litter in forming a protective layer that could insulate the soil microbial communities from cold winter weather.

5.5.2.4 Priming from Litter Additions

Contrary to some studies (e.g. Kuzyakov *et al*., 2000), there was no increased respiration from DL plots compared to CON plots. Other studies (Subke *et al*., 2004; Sulzman *et al*., 2005) have reported increases in soil respiration due to 'priming of the soil', whereby increased substrate, increases microbial biomass and decomposition of different sources, which therefore leads to higher CO_2 fluxes. This was not the case here, as reflected by DL often having lower fluxes than CON fluxes and also by no significant difference found in isotopic source signal from both treatments, suggesting the total respiration was contributed by similar soil processes.

There was also little difference between CON and DL respiration and that from NL, suggesting that there was no priming effect caused by litter addition in either CON or DL plots. The reasons for this can be related to the quality of the litter as Kuzyokov *et al*., (2000) found little evidence of a priming effect occurring with recalcitrant litter, such as *Miscanthus* litter. The high C:N Ratio indicates a low N availability so even though there is more substrate and higher N input from the litter (Figure 5.6), this is likely to have been taken up by the *Miscanthus*, leaving little available N for soil microbes to decompose the extra litter. There is also a low soluble C content to the litter compounding this effect. The rate decomposition of the litter from CON and DL

plots was indistinguishable, confirming this result, because if there was priming, higher rates of decomposition would be expected in DL plots.

5.6 Conclusion

Miscanthus, from our site in Lincolnshire showed that the soil respiration was dominated by belowground respiration from the rhizosphere and decomposition of SOM. Our results were comparable with other estimates of rhizosphere respiration and decomposition of above- and below-ground decomposition although litter decomposition estimates in 2009 were lower than reported estimates due to low soil moisture inhibiting decomposition. In addition, there was little evidence of a priming effect due to the high C:N ratio. If this is true for all situations where *Miscanthus* is grown, this will not significantly increase soil respiration with increase litter senescence due to elevated CO_2. This would result in these crops remaining a viable alternative to fossil fuels under future predictions of increased CO_2 in the atmosphere.

5.7Acknowledgements

We would like to thank the farmer Jonathon Wright for access and use land; Simon Oakley, Sean Case, Laura Barringer, Nicola Thompson, Mike Whitfield & Nick Ostle for assistance with trenching and fieldwork; Andy Robertson for useful comments to the manuscript; to Clive Woods and Helen Quirk, Helen Grant for access to laboratory equipment and the Stable Isotope Facility at CEH Lancaster. This work was funded under the CEH Environmental Change Integrating Fund Project no C03746.

Chapter 6

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6. General Discussion

The first aim of this research was to quantify the in *situ* soil greenhouse gas (GHG) budget and to establish the drivers of these GHG fluxes for two UK second generation bioenergy crops, *Miscanthus* and short rotation coppice (SRC) willow. The second aim of this research was to provide a more in-depth understanding of carbon (C) cycling under *Miscanthus* i.e. litter and roots through two field experiments.

6.1 Main Research Findings

6.1.1 Fluxes

The soil GHG budgets from SRC willow (Chapters 2) and *Miscanthus* (Chapter 3) showed that carbon dioxide (CO_2) was the main flux from soils in both these crops, with methane (CH_4) and nitrous oxide (N_2O) contributing very little to the overall soil GHG budget. Although these crops were not directly compared in terms of GHG emissions, the annual budgets showed that, on average, SRC willow had a lower budget (738 GWP) compared to *Miscanthus* (1013 GWP). This may be due to the different way in which these budgets were calculated, with SRC willow budget being calculated by extrapolating the mean annual flux and *Miscanthus* CO_2 fluxes being modelled using continuous air temperature and soil moisture. *Miscanthus* CO_2 fluxes were modelled using regression analyses as there was access to continuous hourly environmental data for this crop only.

CO_2 fluxes from soils in both crops showed a clear seasonal pattern and was highly correlated to temperature, but soil moisture content also influenced fluxes. These correlations with soil respiration have been reported by many other studies (Lloyd &

Taylor, 1994; Bowden *et al*., 1998). A difference in CO_2 fluxes between *Miscanthus* and SRC willow was expected due to differences in the ages and characteristics and management strategies of the crops (SRC willow planted in 2000 and *Miscanthus* planted in 2006). However, it was expected that since SRC willow was fully established by the time GHG measurements were started and *Miscanthus* was not, that fluxes of CO_2 would be higher due to a large belowground live biomass pool. Studies have, however, reported that after the first year of planting, *Miscanthus* can quickly become established (Beale & Long, 1995), with some linking this to the fast establishment to the parent species of *Miscanthus* from native habitats in Japan, being highly productive primary colonisers (Stewart *et al*., 2009; Heaton *et al*., 2010). This may offer some explanation for why Miscanthus CO_2 fluxes were higher.

The summer time fluxes in *Miscanthus* were higher than in SRC willow, and there was limited evidence from Chapter 3 that *Miscanthus* CO_2 flux was limited by a low soil moisture content. The concern that water limitation could affect *Miscanthus* productivity was raised by Richter *et al*., (2008) and was linked to rooting depth. Although roots can grow to a depth of 2 m (Neukirchen *et al*., 1999), Finch and Riche, (2008) suggested that the 'effective rooting depth' was only 1.5 m, which has implications for *Miscanthus* with environmental change. Heaton *et al*., (2010) suggested that *Miscanthus* in warm Mediterranean climates is not viable without irrigation.

There appeared to be little evidence of water supply limiting CO_2 flux in SRC willow, which may be due to the deeper roots allowing SRC willow to dry up soil to a depth of 2 to 3 m (Rowe *et al*., 2009; Crow & Houston, 2004). Also, as highlighted in Chapter 4, that the moss which grows on the ground around the crop, may act as a protective layer and maintain soil surface moisture levels (Garcia-Pausas, 2004).

The methods employed in this study only captured CO_2 fluxes from the soil surface such that only part of the C balance was measured in both crops i.e. net ecosystem exchange (NEE) of CO_2 from the whole system was not quantified. Techniques such as eddy covariance are commonly used to measure CO_2 fluxes between the plant canopy and the atmosphere and uses meteorological data and mathematical algorithms to estimate CO_2 (and other fluxes) over a certain period of time (Baldocchi *et al*., 2003). This technique would have allowed a full GHG balance to be measured and to identify if these crops are GHG neutral. A partner study from Drewer *et al*., (2011) at Lincolnshire, used the eddy covariance technique and showed that the overall GHG balance was negative, showing overall net C uptake and that the uptake was higher for *Miscanthus* than for SRC willow. Unfortunately, the same study did not have the opportunity to use eddy covariance in neighbouring arable fields, so it could not be determined if *Miscanthus* and SRC willow had a preferential GHG balance compared to the previous land use type.

The annual budget of CH_4 for both *Miscanthus* and SRC willow showed that SRC willow was a slight sink for CH_4 and *Miscanthus* was a weak source of CH_4. This may be partly due to the difference in the management of these crops, with *Miscanthus* being harvested on an annual basis and SRC willow being harvested on a 3-yearly cycle. Methanotrophic communities are known to be negatively affected by disturbance and compaction events (Hütsch, 2001; Smith & Conen, 2004) and since *Miscanthus* is harvested more often, potentially less CH_4 oxidation was occurring in the soil under the *Miscanthus*. Overall, CH_4 oxidation rates were low under both crops and this is consistent with the previous land use being in an arable rotation with N fertiliser, a known inhibitor of oxidising bacteria (Mosier *et al*., 1991).

Both *Miscanthus* and SRC willow emissions of CH_4 were shown to increase with increasing soil moisture or water-filled pore space (WFPS), an effect that is well known to cause anaerobic conditions promoting methanogensis and has been reported by a number of other studies (Bowden *et al*., 1998; Schimel Gulledge, 1998; Hütsch, 2001).

In general, the annual budget showed both soils to be a net weak source for N_2O emissions but fluxes were minimal. This was unsurprising since neither of these crops were fertilised, and the nitrogen (N) availability in the soil was likely to be minimal due to the high N efficiency of these crops (Kadvir *et al*., 2008). The results seem to indicate a link to high soil moisture content and WFPS (above 60%) leading to higher N_2O emissions, suggesting that the process of denitrification may responsible.

The results from this work regarding soil GHG emissions are comparable to a limited number of other studies investigating CH_4 and N_2O from these crops (Jørgensen *et al*., 1997; Hellebrand *et al*., 2003; Kavdir *et al*., 2008; Gauder *et al*., 2011; Drewer *et al*., 2011). Crucially, emissions of CH_4 and N_2O were found to be lower than first generation crops such as maize or wheat, which confirms why there is continued interest and use of second generation crops over first generation crops. First generation crops tend to have high N_2O emissions, which are a key sustainability issue due to the high global warming potential of N_2O compared to CO_2. This is important from a UK perspective because of the planned increase use of SRC willow and *Miscanthus* for bioenergy production in meeting renewable energy targets and national GHG reduction targets. The way in which these CH_4 and N_2O fluxes are controlled, which is partly through management practices (soil disturbance and lack of fertiliser), offers useful information on possible ways to keep emissions of these gases to a minimum.

The minimal fluxes of CH_4 and N_2O is also encouraging for new technologies that are emerging which are designed to use second generation crops for the production transport fuels. These second generation crops would offer better GHG savings than first generation crops. The move to second generation crops will also help to reduce the other associated negative impact of first generation crops, which is competition for land with food crops. Studies carried out with SRC willow have also demonstrated the ability of SRC willow to withstand heavy metal pollution and can therefore be used as a way to remediate contaminated sites (Vervaeke *et al*., 2003).

6.1.2 Carbon cycling

One of the key reasons for lower N_2O emissions from SRC willow and *Miscanthus* is the reduced need for fertiliser due to the efficient of nutrients of both these crops. Evidence from this work would suggest that the input of litter is important in returning nutrients to the soil surface. There have been several papers that suggest the use of crop residues from major crops such as maize, wheat, barley, sorghum and sugar cane etc., in bioethanol production (Kim & Dale, 2004; Sommerville, 2006). The results from Chapter 4 and 5 would suggest that residue removal could reduce nutrients and C input to the soil and could affect soil moisture content, which could have further impacts on soil processes. With continued residue removal, nutrients would have to be added to the soil using either as fertiliser or manure, which could have serious consequences to the GHG balance of these crops, through increasing N_2O emissions as well as extra cost. Blanco-Canqui and Lal (2007) found that removing crop residues from a maize crop had negative effects on SOC accumulation, soil productivity, plant available water reserves and ultimately plant yield. The high yielding capability of these crops is one of the reasons why they are used as a bioenergy crop. Removing

residues would seem to have numerous negative effects that may deem these crops unsustainable.

Litter is also important in C sequestration in these crops. Chapter 4 identified that a high C:N ratio of *Miscanthus* litter and high lignin content on SRC willow litter were the main controlling factors of decomposition. The rate of decomposition resulted in a visible build up of litter on the soil surface in both crops, most notably in *Miscanthus*. Although this was not directly measured in the work, other studies have noted this accumulation and the contribution of this to SOM (Beale & Long, 1997; Beuch *et al*., 2000; Kahle *et al*., 2001). The contribution of *Miscanthus* derived C from litter to soil C was also found in Chapter 5 of this study, further confirming the importance of litter in this crop.

6.2 Future Research

The need for more field studies has been a recurring feature within this discussion. There have been very few published data regarding GHG for these crops in the UK and it is important that more UK field-based studies are carried out to understand the full impact of SRC willow and *Miscanthus* in different climatic and soil conditions. This is critical given the commitment the UK has given to reducing national GHG and the incorporation of bioenergy into the legislation such as the Energy Act 2011 to help deliver, in conjunction with other renewable energies, these national GHG savings. There are now such emerging studies through projects such as the ETI (Energy Technologies Institute) ELUM (Ecosystem land-use Modelling) project. Here, annual GHG budgets are being produced over a two-year period for *Miscanthus*, SRC willow, short rotation forestry, oil seed rape, maize and grassland plots at five locations in the

UK including the Lincolnshire site. The measurement techniques and sample methods used within this work and presented here have been adopted within the ELUM project. Ultimately, data from field-studies needs to be fed into soil C models to be able to parameterise and validate the outputs, and so improve our predictions for the most sustainable bioenergy deployment. Data from this current work at Lincolnshire are being used to parameterise the models ECOSSE and DayCent focussing on how bioenergy crops impact on soil C stocks and GHG emissions.

This study highlighted the importance of litter for providing nutrients, increasing C sequestration through slow decomposition rates and forming a protective layer on the soil surface, which can buffer the soil from large changes in temperature and moisture. More research is needed to confirm these results and better understand the role that litter plays in these crops. Chapter 4's litter decomposition study focussed on environmental and litter quality effects on governing the rate of litter decomposition. Decomposer organisms are involved in this process (but have had little investigation) and could be a possible route of future research. As well as identifying the main groups of decomposer organisms (microbial, fungi or macro-invertebrates) involved in litter decomposition, the way in which they respond to changes in environmental conditions and in turn how this effects decomposition rates could be investigated. This could be linked in to the contribution of litter decomposition to overall soil respiration and whether increasing temperatures will result in higher decomposition rates or if increased temperatures will limit decomposition rates by limiting soil moisture?

At the Lincolnshire field-site there has been no investigation into the quantification of belowground biomass. Studies have shown that both these crops have extensive rooting systems (*Miscanthus* - Neukirchen *et al*., 1999, SRC willow – Crow &

Houston, 2004) but so far most of the attention has been related to bulk soil respiration and aboveground litter inputs and decomposition. Since the manipulation study highlighted that approximately 75% of total soil respiration was from belowground processes, (the other 25% from litter decomposition), it would seem sensible that this is one of the next areas to be researched. The amount of belowground biomass production indicates how much C is allocated belowground and changes in biomass can be linked to changes in C allocation over a growing season. Ultimately, belowground biomass can be used with aboveground biomass and soil respiration to determine a full C balance of a crop and soil system. However, there is difficultly in actually carrying out such measurements due to the large errors that can be associated with belowground biomass estimates (Toma *et al*., 2010a). A large number of samples need to be obtained to account for the special variability in rooting and rhizome distribution.

6.3 Overall Conclusion

Overall, the results from this study confirms previous findings that there are minimal emissions of CH_4 and N_2O from soil in second generation crops of SRC willow and *Miscanthus*. CO_2 flux was found to be the major efflux from soil and in *Miscanthus*, the majority of this flux was derived from belowground processes. Litter played an important part in providing nutrients to the soil, which are vital in systems that are not fertilised. Litter also contributed to SOM accumulation on the soil surface and may provide long-term C sequestration. Overall, the results from this study, combined with other literature would suggest that these crops offer advantages over first generation crops but more field-based studies are required in the UK to be able to say if these crops can offer large-scale GHG savings needed from this renewable energy source.

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