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Ecosystem Land Use Modelling & Soil C Flux Trial (ELUM)

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Report on Bioenergy Crop Management and Land-use Change

REPORT

V1.2

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Jon Finch[1], Rachel Marshall[1], Dafydd Elias[1], Emily Clark[1], Julia Drewer[1], Ross Morrison[1], Jonathan Oxley[1,2], James Morison[3], Mike Perks[3], Lukas Siebicke[3], Matt Wilkinson[3], Sirwan Yamulki[3], Iain Donnison[4], Kerrie Farrar[4], Alice Massey[4], Jon McCalmont[4], Giorgio Alberti[5,6], Zoe Harris[5], Gail Taylor[5] and Niall McNamara[1].

[1]Centre for Ecology & Hydrology

[2] Energy Technologies Institute

[3] Forest Research

[4] IBERS, Aberystwyth University

[5] School of Biological Sciences, University of Southampton

[6] Department of Agriculture and Environmental Science, University of Udine

EXECUTIVE SUMMARY

The ELUM project aim has been to develop a model to quantitatively assess changes in the levels of carbon in soil, combined with the GHG flux which results from the conversion of land to bioenergy crop production. This deliverable reports the findings from one component of the ELUM project which was designed to provide detailed measurements of soil organic carbon (SOC) and greenhouse gas (GHG) fluxes in order to calibrate and test the model.

Research Highlights

- Long-term monitoring of field sites demonstrated that all the land covers were carbon sinks, with the exceptions of *Miscanthus x Giganteus* at Aberystwyth during the year of conversion, and the grass reference site at West Sussex.
- In general, fluxes of microbial respired CO_2 were found to be lower in the bioenergy sites compared to either the grass or arable reference sites.
- Fluxes of CH_4 and N_2O were shown to be close to negligible across all bioenergy land-uses; significant reductions in N_2O emissions were associated with land-use change from arable to perennial bioenergy crops.
- Greater accumulation of carbon was observed with *Miscanthus x Giganteus* when compared to SRC willow. This can partially be attributed to differences in stage of growth phase but may also indicate greater overall carbon-use efficiency of *Miscanthus x Giganteus*.
- Between the different *Miscanthus* genotypes there were no discernible differences in the allocation of recently assimilated C (through photosynthesis) to the soil, nor differences in C losses through plant and soil respiration.

There is a lack of quantitative information on the change in greenhouse gas fluxes and soil organic carbon for land-use change (LUC) to second-generation bioenergy crops with respect to historical land covers (arable, grass and woodland). This report describes a programme of GHG measurements, made in the UK under Work Package 3 (WP3) within the ETI's Ecosystem Land Use Modelling Project ("ELUM"), to contribute to filling this information gap and delivering quantitative data for model development in Deliverable 4.3 (PM07.4.3_WP4_LUC and Crop Management Model).

A network of sites was established consisting of four UK locations: Aberystwyth (*Miscanthus x giganteus* and grass fields, and trial plots of *Miscanthus* varieties); East Grange, Fife (short rotation forestry (SRF), short rotation coppice (SRC) willow, grass and arable); Lincolnshire (*Miscanthus x giganteus*, SRC willow and arable) and West Sussex (SRC willow and grass). Measurements were made, over a two-year period, of the soil carbon (C) and soil GHG emissions at the 12 sub-sites along with continuous measurements of meteorological conditions. In addition, eddy covariance

(EC) measurements of net ecosystem exchange (NEE) were made at seven of the sub-sites. Pulse labelling experiments were carried out to quantify the C assimilation of two biomass crops at the Lincolnshire site and for three *Miscanthus* genotypes at the Aberystwyth site. The development of novel technologies of measuring GHG fluxes was also part of this work programme, but these activities are covered in a separate report – deliverable D3.4 (BI1001 PM07.3.4 WP3 Report on Novel GHG Techniques).

EC measurements were used in order to determine whole-system carbon balances. Measurements of NEE were made, as 30-minute averages, over the main land cover types: *Miscanthus x giganteus* (Aberystwyth and Lincolnshire), SRC willow (Lincolnshire and West Sussex), SRF (East Grange), grass (West Sussex) and arable (Lincolnshire). The data showed that all the land covers were sinks of C, except the *Miscanthus x giganteus*, at the Aberystwyth site during the year of conversion, and the grass at the West Sussex site. NEE was partitioned into plant C uptake (gross primary productivity (GPP)) and ecosystem C losses through plant and soil respiration (total ecosystem respiration (TER)). The SRF site had the highest GPP of all land covers, with associated low TER resulting in the forestry site acting as the most efficient C sink. The GPP of the two fields of *Miscanthus x giganteus* were similar, but the total ecosystem respiration (TER) was much lower at the Lincolnshire site than at the Aberystwyth site, possibly due to the difference in time since conversion. In comparison, the annual TER for the SRC willow at the Lincolnshire and West Sussex sites was similar, but the annual GPP at the West Sussex site was much higher.

Chamber-based GHG measurements were used to determine the contribution of CO_2 and non-CO_2 gases (CH_4 and N_2O) to soil GHG emissions under bioenergy crops. The aim was to monitor potential reductions in the emissions of soil GHGs following transition, particularly with regard to the arable to bioenergy transition. Overall fluxes of CH_4 and N_2O were shown to be close to negligible across all bioenergy land-uses. Potential benefits for the reduction of N_2O emissions following a switch from arable crop to woody, perennial bioenergy crops were observed. This is most likely linked to reductions in fertiliser application following this transition and therefore management of the bioenergy crop will be important in determining whether valuable reductions in N_2O are obtained. CO_2 fluxes were partitioned into microbial (heterotrophic) respiration and plant/root (autotrophic) respiration using partitioning factors taken from the literature and from field-based trials where these were available. In general, heterotrophic CO_2 production from soils under bioenergy was lower, which suggests that microbial turnover of C is reduced in these bioenergy systems. However, there is still much uncertainty with regard to partitioning of soil respiration into auto- and heterotrophic components. We recommend that future research focus should be on determining the relative contributions of plant and microbial respiration to total soil CO_2 flux under different land-use scenarios.

In addition to the GHG measurements made across the network sites, the dynamics of C flow in *Miscanthus* and SRC willow were examined using a ^{13}C pulse labelling technique. Pulse labelling experiments were performed to examine the turnover and

allocation of recently fixed photosynthate. Large, tent-like chambers were used to create a ^{13}C-CO_2 enriched atmosphere around the vegetation, thus introducing ^{13}C into the biomass which could be traced into different plant structures, the soil, the microbial biomass and into respired CO_2.

At the Lincolnshire site recently fixed C was rapidly turned over in the leaves of both *Miscanthus x giganteus* and SRC willow with up to 50% lost within 15 hours of the pulse. These initial losses can be attributed to a "fast" C pool with rapid turnover through leaf and soil respiration. The remaining fraction of ^{13}C becomes incorporated into a much slower "structural biomass pool" with C supporting growth and being locked into above- and below-ground structural components or being re-allocated into short- and long-term storage. A greater proportion of the recently fixed ^{13}C appears to be retained in the "structural biomass pool" of *Miscanthus x giganteus* compared to the SRC willow. This can partially be attributed to differences in stage of growth phase but may also indicate greater overall carbon use efficiency of *Miscanthus x giganteus*.

Allocation and turnover was examined in three *Miscanthus* genotypes at the Aberystwyth sub-site C. The level of ^{13}C enrichment decreased from above-ground vegetation to rhizome to root and into the soil, with this pattern being observed for all genotypes. Differences in C allocation and above-ground morphology between the genotypes were not found to impact on total soil respiration nor ^{13}C allocation to the soil. This reflects what was observed during the two years of soil respiration measurements at this site.

In summary, the findings of this work package suggest that bioenergy crops are expected to have a largely negligible impact on emissions of non-CO_2 GHGs, with potential benefits with regard to N_2O emissions when transitions from arable to bioenergy are observed. With regard to CO_2, decreases in microbial respiration were observed from the majority of transitions to bioenergy but there is uncertainty regarding the partitioning of CO_2 fluxes into the hetero- and autotrophic components. Arable and bioenergy crops showed net C uptakes when measured by EC with the strongest C sink being the SRF. EC over the grass control in West Sussex demonstrated that it was a source of C. Caution should be applied when drawing conclusions from one site in isolation and the overall conclusions regarding the GHG benefits of transitions should be drawn from the model rather than the data reported in Deliverable 3.5.

The deliverable description and acceptance criteria for this report are as follows:

Deliverable D3.5:	Final detailed technical report on effects of LUC and subsequent bioenergy crop management on changes in dynamic soil carbon and GHG for the UK across a range of soil types and climatic conditions. The report will also describe the mechanisms resulting in these changes in soil carbon and GHG for LUC and subsequent recommendations on Bioenergy crop management. All papers generated from this work package (either published, accepted or submitted) must be provided in the appendix.
Acceptance Criteria:	A final report detailing all WP3 results from the three year study. An executive summary must be provided to give an overview of WP3. The report must include all field soil and GHG sampling with full statistical analyses. Results will be presented in tables and graphs with full statistical analyses. A concluding section will review all results and a section will describe future research requirements post project.

REFERENCES TO OTHER ELUM REPORTS

The reader's attention is drawn to the following additional ELUM reports which are referred to in this report:

- PM01.2.1_Chronosequence Report
- PM04.2.2_WP2 Year 1 Chronosequence Report
- PM06.2.3_WP2 Year 2 Chronosequence Report
- PM04.3.2_WP3 Year 1 Report
- PM06.3.3_WP3 Year 2 Report
- PM07.3.4_WP3_Report on Novel GHG Techniques
- PM07.4.3_WP4_LUC and Crop Management Model

In addition, a glossary of standard terms and abbreviations used in this project is shown in Appendix 3.

CONTENTS

1. INTRODUCTION

Agricultural land-use change (LUC) affects the soil organic carbon (SOC) balance (Jenkinson *et al.,* 1990; Coleman *et al*., 1997) and greenhouse gas (GHG) fluxes (Dobbie *et al*., 1996). Consequently, LUC to perennial bioenergy crops may be beneficial to ecosystem services (Hiller *et al*., 2009; Lemus and Lal, 2005; Kavdir *et al*., 2008). For example, increased SOC under bioenergy crops will improve soil quality (regulating service) whilst reduced GHG emissions will improve the climate (regulating service). However, the evidence is limited on how different types of bioenergy crop, especially second-generation energy crops, will affect SOC and the net mitigation benefit.

Quantitative information on the change in GHG fluxes and SOC is needed for LUC to energy crops with respect to historical land covers (arable, grass and woodland) and current changes of land-management practices (fertiliser, harvest). The resilience dynamics and SOC stock level, as well as the GHG budgets, require long (>10 yrs) SOC and medium-term (2-5 yrs) of flux measurements to account for such effects.

While the assessment of LUC and management practices on the soil carbon (C) stock is essential, it is equally important to quantify the response of GHG exchanges, for example, CO_2, N_2O and CH_4 (Robertson *et al*., 2000 and Smith *et al*., 2001). To date, much of the GHG information that has been used to produce bioenergy crop life-cycle analyses (LCA) has been based on GHG assumptions rather than robust data. In a review of 44 life-cycle analysis studies of first- and second-generation biofuels, Whitaker *et al.,* (2010) highlighted that the most uncertain aspects in biofuel production LCAs relate to soil GHG (e.g. N_2O) emissions. On a molecule-for-molecule basis, N_2O has a global warming potential that is 296 times that of CO_2 on a 100-year time scale. Therefore it is suggested that soil N_2O fluxes can strongly influence the extent to which bioenergy crops are sustainable relative to fossil fuels (Adler *et al*., 2007; Crutzen *et al*., 2008). As nitrogen (N) inputs through fertilisation of the soil are the primary driver of N_2O releases to the atmosphere, there may be potential benefits of switching from high N-input first-generation (e.g. wheat bioenergy crops) to lower N-input second-generation bioenergy crops (e.g. *Miscanthus*). CH_4, like N_2O, is a potent GHG (23 times greater than CO_2 during a 100-year lifetime), and so also needs to be fully accounted for when calculating net GHG fluxes from bioenergy crops. The largest terrestrial sink for CH_4 (i.e. a net decrease in atmospheric CH_4) is via biological consumption in soil, and N inputs have been demonstrated to diminish this sink-strength substantially over the short to medium term (Dobbie and Smith, 1996). Potentially, second-generation perennial crops systems may promote biological soil CH_4 consumption through providing stabilised, no-till soil conditions. Conversely, the disruption of soil through tillage and ploughing disrupts the soil CH_4 sink. The potential GHG benefits of different cropping systems and their associated management interactions need to be fully addressed. Overall, care must be taken when discussing bioenergy sustainability issues until we generate long-term experimental data that encapsulate varying climatic conditions and management interventions. New studies should aim to provide a simultaneous accounting of the net exchanges of CO_2, N_2O and CH_4 in a range of bioenergy crop

types. This opens the possibility to calculate the budget, per unit area, of soil GHG exchanges in CO_2-C equivalents. It is these issues that WP3 addressed by making available empirical data to provide an evidence basis for the modelling activities of WP4.

The main activity in WP3 was the measurement of the soil C and soil GHG emissions at 12 sub-sites across the UK during a two-year period; these sub-sites comprised 11 commercial-scale bioenergy field-sites and one set of trial plots of *Miscanthus* varieties. These measurements are described and analysed in Section 4. The sub-sites were located at four sites, which are described in Section 2. Eddy covariance (EC) measurements of net ecosystem exchange (NEE) (i.e. whole system carbon fluxes) were made at seven of the sub-sites and are described and analysed in Section 3. In addition, pulse-labelling experiments were carried out to quantify the C assimilation of two biomass crops, which are described in Section 5. The development of novel technologies of measuring GHG fluxes was also part of this work programme but these activities are covered in deliverable report D3.4 (BI1001 PM07.3.4 WP3 Report on Novel GHG Techniques).

The deliverable and acceptance criteria for this report are defined as:

Deliverable D3.5:	Final detailed technical report on effects of LUC and subsequent bioenergy crop management on changes in dynamic soil carbon and GHG for the UK across a range of soil types and climatic conditions. The report will also describe the mechanisms resulting in these changes in soil carbon and GHG for LUC and subsequent recommendations on Bioenergy crop management. All papers generated from this work package (either published, accepted or submitted) must be provided in the appendix.
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2. THE SITES

WP3 measurements were conducted at a network of four sites that are a mix of commercial and experimental operations. These are located in England, Scotland and Wales (Figure 2.1) and included five land cover types (Table 2.1). A summary of the soils, annual rainfall and previous land use is given in Table 2.2. Due to resource constraints the project did not attempt to follow the transition from one land cover to another, with the exception of Aberystwyth sub-site A. Instead, measurements were made on existing land covers in order to quantify the differences in soil C and GHG emissions to inform the modelling in WP4, which has simulated the transitions. For example, at the Lincolnshire network site, measurements informed the transitions from arable to *Miscanthus x giganteus* or short rotation coppice (SRC) willow, *Miscanthus x giganteus* to arable or SRC willow, and SRC willow to arable or *Miscanthus x giganteus*.

Two of the sites, East Grange, Fife (maintained by Forest Research - FR) and Lincolnshire (maintained by the Centre for Ecology & Hydrology - CEH) existed before the ELUM project began and existing measurements were augmented by the ELUM project. Soil GHG measurements were not being made at either of the sites, although some had been made in the past at the Lincolnshire site. At FR's East Grange site, EC measurements were being made over short rotation forestry (SRF), funded by FR's core science. At the Lincolnshire site, EC measurements were being made on a commercial farm, over *Miscanthus x giganteus* and SRC willow, funded by CEH's National Capability which is provided by the UK's Natural Environment Research Council (NERC). The ELUM project funded about half the instrument purchase cost and covered installation costs to enable the establishment of a third EC system in an adjacent arable field.

Aberystwyth sub-sites A and B are newly established on a grass field, part of which was converted to *Miscanthus x giganteus* as part of the University's research programme. Funding for part of the measurements at this site has come from the NERC Carbo-Biocrop research grant. West Sussex is a new site, on a commercial farm, and was established by the University of Southampton on grass and SRC willow.

These sites achieve the aim of covering a range of "conventional" land uses (e.g. arable, grassland), second-generation bioenergy crops, climates and soils. Figure 2.2 illustrates the time periods of measurements of EC and soil GHG chambers made at the sites and that were made available to WP4.

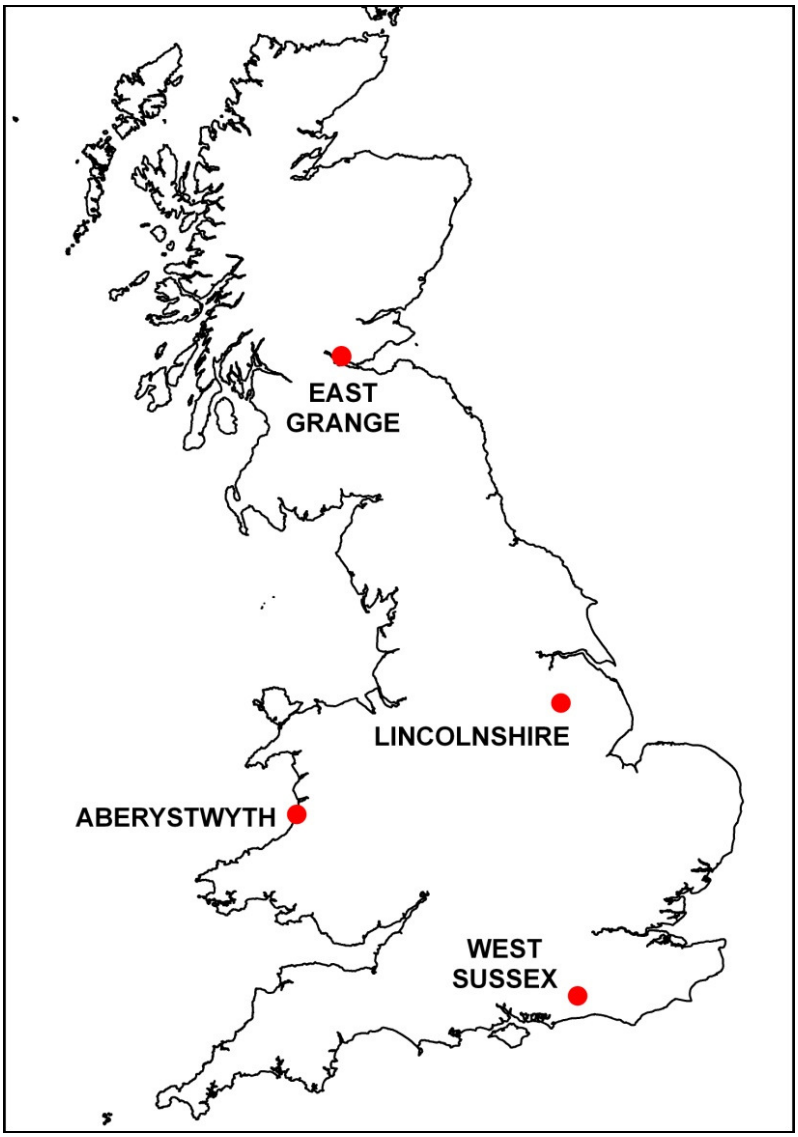

Figure 2.1: Location of the network sites

Table 2.1: The location of and the start dates of measurements at the network sites

Network site	Sub-site	Land use	Latitude	Longitude	Start month of soil GHG* flux measurements	Eddy covariance start date
Aberystwyth, West Wales	A	*Miscanthus x giganteus*	52°25'17" N	4° 04'14" W	Dec-2011	03-Jan-2012
	B	grass	52°25'17" N	4° 04'14" W	Dec-2011	none
	C	*Miscanthus* genotype trial plots	52°24'06" N	4° 02'12" W	Nov-2011	none
East Grange, Fife	A	SRF	56°05'19.4" N	3°37'33.1" W	Jan-2012	01-Oct-2011
	B	grass	56°05'19.4" N	3°37'33.1" W	Jan-2012	none
	C	SRC willow	56°04'58.8" N	3°37'11.0" W	Feb-2012	none
	D	arable	56°04' 48.0" N	3° 37' 37.6"W	Apr-2012	none
Lincolnshire	A	*Miscanthus x giganteus*	53°19′ 11.8″ N	0° 35′ 15.4″ W	Nov-2011	07-May-2008
	B	SRC willow	53°19′ 11.2″ N	0° 35′ 03.3″ W	Nov-2011	13-Oct-2009
	C	arable	53°19′ 19.3″ N	0° 35′ 04.3″ W	Nov-2011	04-Apr-2012
West Sussex	A	SRC willow	50°58’49.3” N	0°27’03.7” W	Nov-2011	16-Apr-2012
	B	grass	50°58’35.3” N	0°27’20.9” W	Nov-2011	22-Nov-2012

* GHGs measured were CO_2, CH_4 and N_2O

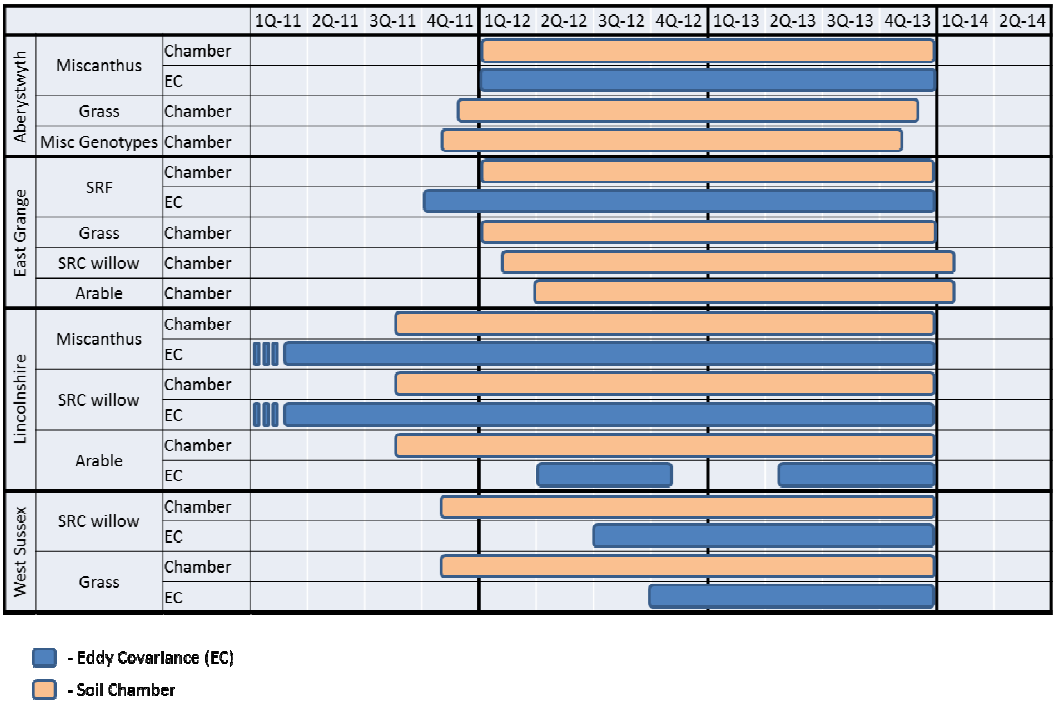

Figure 2.2: The time periods of EC and soil GHG chamber measurements made at the sites. (NOTE – the dashes at the start of two of the EC measurements at the Lincolnshire site indicate that measurements began before the ELUM project started. The gap in the EC coverage from the arable sub-site is due to measurements not being made because of land management operations)

Table 2.2: Soils, rainfall and previous land use of the network sites

Network site	Mean annual rainfall (mm)	Sub-site	Land use	Soil description	Land use prior to energy crop planting	Year of conversion to energy crops
Aberystwyth	1075	A	*Miscanthus x giganteus*	Freely-draining slightly acid loamy soils	semi-improved perennial ryegrass	2012
		B	permanent grassland	Freely-draining slightly acid loamy soils	n/a	n/a
		C	*Miscanthus* genotype trial plots	Well-drained fine loamy and fine silty soils over rock	grass	2010
East Grange	773	A	SRF	Predominantly surface-water gley, indurated, cultivated phase (7xc) soil with a high proportion of silt	predominately barley with occasional fodder grass cropping	2009
		B	rotational grassland	Predominantly surface-water gley, indurated, cultivated phase (7xc) soil with a high proportion of silt	n/a	n/a
		C	SRC willow	A mixture from gleyed cultivated brown earth to 1(g)c to surface-water gley, indurated, cultivated phase (7(x)c) soil with a high proportion of silt	predominately barley with occasional fodder grass cropping	2009
		D	arable	A mixture from gleyed cultivated brown earth to 1(g)c to surface-water gley, indurated, cultivated phase (7(x)c) soil with a high proportion of silt	n/a	n/a
Lincolnshire	613	A	*Miscanthus x giganteus*	Slowly permeable seasonally waterlogged fine loamy soils	Rotation of arable crops: 2-3 years winter wheat followed by OSR as a break crop	2006

Network site	Mean annual rainfall (mm)	Sub-site	Land use	Soil description	Land use prior to energy crop planting	Year of conversion to energy crops
		B	SRC willow	Slowly permeable seasonally waterlogged fine loamy soils	Rotation of arable crops: 2-3 years winter wheat followed by OSR as a break crop	2000
		C	arable	Slowly permeable seasonally waterlogged fine loamy soils	n/a	n/a
West Sussex	827	A	SRC willow	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils	set-aside	2008
		B	permanent grassland	Slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils	n/a	n/a

2.1 Aberystwyth, West Wales

Sub-sites A and B were chosen to quantify the changes in soil C and GHG emissions during the process of conversion from conventional agriculture to a biomass crop (*Miscanthus x giganteus* in this instance). They are located in a field of 7 ha (Penglais) which had been under management as semi-improved grazing, for both cattle and sheep; it was last ploughed and sown with a perennial ryegrass ley in 2006. The majority of the field was converted to *Miscanthus x giganteus* (sub-site A) but two areas, one at the eastern end of the field and the other at the western, were retained as grass to serve as references (sub-site B). On 15 March 2012 6 ha of the grass (sub-site A) was sprayed with glyphosate (br. Glyphogan) at 5 l ha^{-1} to kill off the grass; this was subsequently ploughed on the 4 April. Commercial planting by International Energy Crops (Market Drayton, Shropshire) took place on 24 April with a power harrow running in front of the planting machine. A mix of pre- and post-emergent herbicides (Cadou Star @ 0.85 kg ha^{-1} and Glyphogan @ 2 l ha^{-1} respectively) were applied on 17 May 2012. The crop was topped in March 2013 and left on the field. In April a weed control herbicide was applied. This consisted of: Glyphosate (2 kg ha^{-1}), Chlortoluron (1.86 kg ha^{-1}) and Diflufenican (0.068 kg ha^{-1}).

Sub-site C was used for a study to quantify the carbon balance of different *Miscanthus* genotypes and to compare their C sequestration potential, with particular focus on how above-ground plant morphologies might be impacting below-ground activities. It is a trial, established by the University, where various genotypes of *Miscanthus* were planted in 2010. Each plot is 25 m^2 with each genotype in triplicate and agricultural management practices consistent throughout.

At sub-site C, in the first year, four genotypes (*Giganteus (Gig)*, *Sacchariflorus* (Sacc1), *Sinensis (Sin1*), *Sinensis*2 (*Sin2*) were selected and assessed for GHG emissions. These were assessed along with reference plots where *Miscanthus* had not been planted. These particular genotypes were chosen for a variety of reasons. *Gig and Sin1* were chosen for their renowned commercial use and study. *Sacc1* was chosen for its physical differences to *Gig* and *Sin1* in order to get a better understanding of how above-ground features impact below-ground activity. The *Sin*2 was chosen after viewing the field and interpreting its successful establishment as a potentially high-yielding crop. All these plots had the static chambers in place and were part of the GHG measurement recording since November 2011. In the Spring of 2012, another two genotypes (*Sacc*2 and *Sacchariflorus/Lutarioparius* (*Sac/Lut*)) were chosen as better competitors to *Gig* (with regard to harvest yield), and measurements were made from May 2012 onwards. From earlier results it was not deemed necessary to record N_2O and CH_4 data for the two newer genotypes, however, monthly CO_2 fluxes using an IRGA were recorded.

Meteorological conditions at the site, during the period of measurements, are illustrated in Figure 2.3. There were major differences in the monthly total rainfalls between those in 2012 and those in 2013. The long-term average rainfalls were exceeded in 6 months during 2012 but only once, May, during 2013. The monthly average air temperatures during 2012 were generally close to or below average; the exception being March. In the first half of 2013, the monthly average air temperatures continued to be below the long-term average but, in

contrast, during the second half of the year they were above or close to the long-term average.

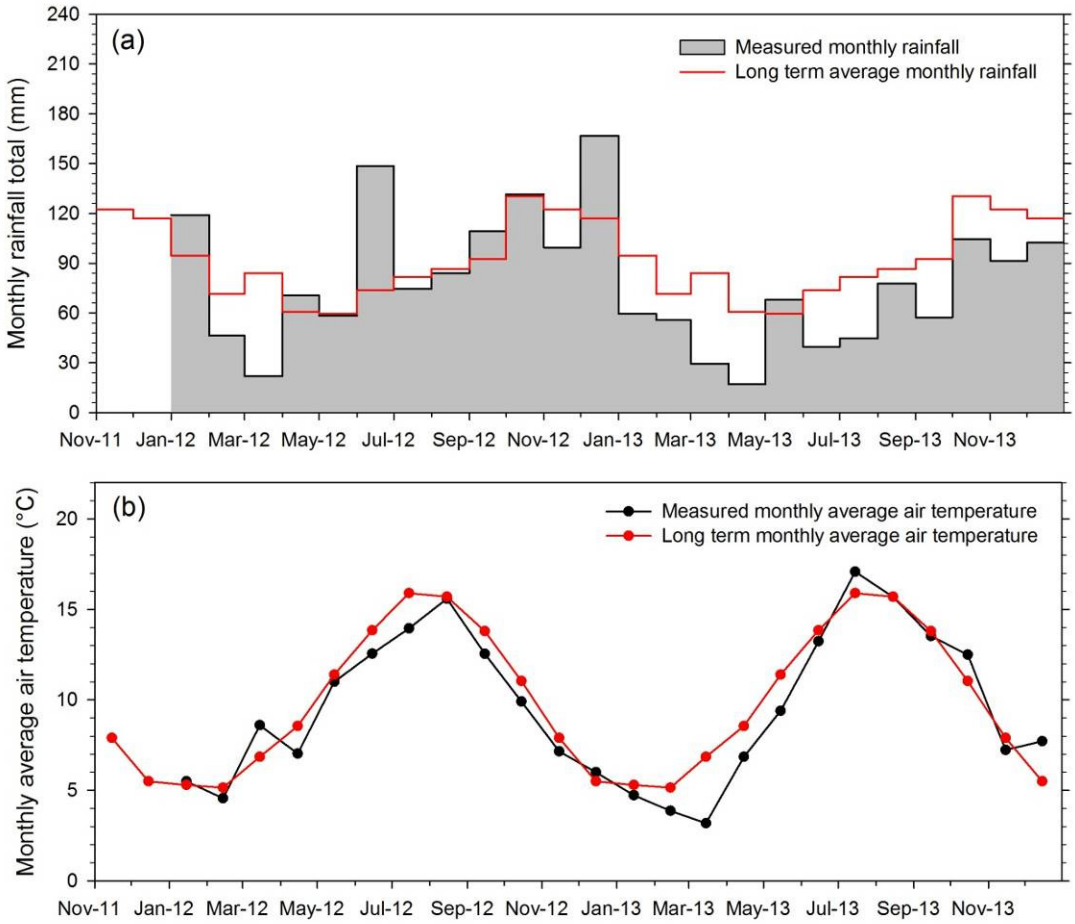

Figure 2.3: Measured monthly rainfall totals (a) and monthly average air temperature (b) compared to the long-term (1981-2010) averages for the Aberystwyth field site.

2.2 East Grange, Fife

At sub-site A, the SRF was planted in February 2009 as bare root stock Scots Pine at a density of 3400 cuttings ha^{-1}; with a mean in-row spacing of 1.6 m and mean between-row spacing of 1.9 m. The preceding land use was a rotation of barley with grassland. Sub-site B is the grass headland around the edge of the plantation.

The SRC willow in sub-site C was planted in May 2009 and covers 34.07 ha. The varieties planted were: Sven, Tordis, Inga and Tora (*Salix viminalis* x *Salix schwerinii*). Different mixes were used in each of the four areas that make up the total acreage. The mean in-row spacing was 0.6 m and the mean between-row spacing was 1.1 m. The preceding land use was a rotation of barley with grassland.

Sub-site D is located in a field on a commercial farm adjoining sub-site C. The field was planted with spring barley in 2013 with 30 t ha^{-1} of farmyard manure applied in February 2013, followed by ammonium nitrate additions on the 30th April (50 kg ha^{-1} of N) and 29th May (50 kg ha^{-1} of N). In 2012 the field was under spring barley with similar additions of fertiliser applied, although a record of the application dates is not available.

Meteorological conditions at the site, during the period of measurements, are illustrated in Figure 2.4. During 2012, monthly rainfall totals higher than the long-term averages were recorded in seven months. Large totals were recorded in July and December; 2.3 and 2.2 times the long-term average respectively. In contrast, during 2013, the monthly rainfall totals were less than the long-term averages in all months except in April, May, July and November. During 2012, the monthly average air temperatures were generally below the long-term averages whilst, in 2013, the air temperatures were close to the long-term averages except in March, April, November and December.

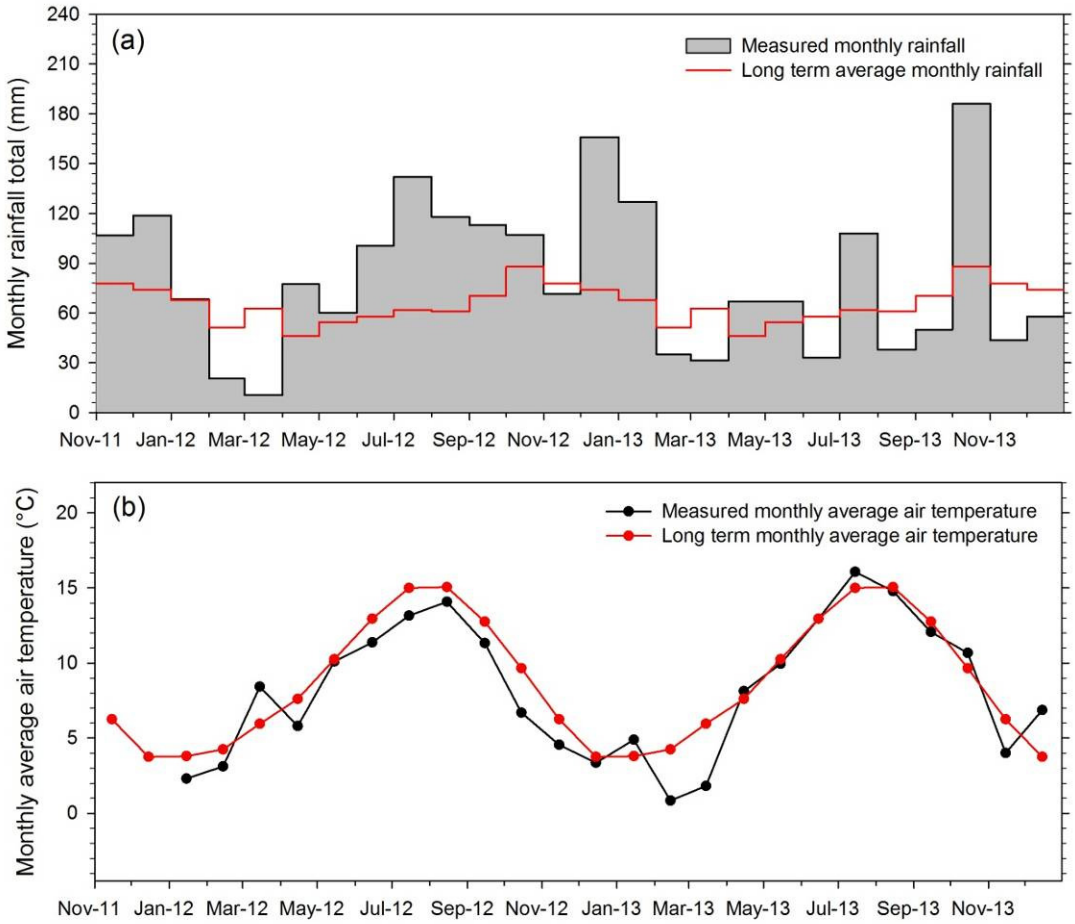

Figure 2.4: Measured monthly rainfall totals (a) and monthly average air temperature (b) compared to the long-term (1981-2010) averages for the East Grange field site.

2.4 Lincolnshire

At sub-site A, the *Miscanthus x giganteus* was planted in spring 2006 covering an area of 11.56 ha. The preparation for the planting was very thorough and consisted of: ploughing, application of Roundup™ (a glyphosate weedkiller), power harrowing and flexi-tine. About 30% of the *Miscanthus x giganteus* had poor survival, mainly in the southern part of the field, and so this was mechanically replanted on 10 May 2007. Some infilling by hand was done in the rest of the field. No fertiliser was used until after the harvest in 2010 when Fibrophos™ (P and K only fertiliser) was applied at a rate of 660 kg ha^{-1}. The yields of the annual harvests are given in Table 2.3. On 17 April 2013 the field was heavily harrowed in order to

improve the yield by cutting up and spreading the rhizomes. In June 2013, chipped wood-waste was spread on to the field.

Table 2.3: Harvest yields of *Miscanthus x giganteus* at the Lincolnshire field site

Date of harvest	Yield (t ha^{-1})*
April 2009	5.7
8 April 2010	10.7
April 2011	9.7
26 April 2012	9.1
Mid-March 2013	10.1

*dry tonnes at the farm gate

At sub-site B, the SRC willow was planted in 2000 and the area of the crop in the field is 9.43 ha. Genetic analysis by Chris Barnes (Warwick University) identified the varieties as Tora (*Salix viminalis* x *Salix schwerinii*), Jora, Jorunn (*Salix viminalis*), Orm, Ulv and Rapp. The preparation for the planting was very thorough: ploughing and application of Roundup™, power harrowing and flexi-tine. The second harvest, after 3 years, was in late Oct/early Nov 2007 and yielded 26.0 t ha^{-1}. It was followed by subsoiling between the rows but this went very close to the stools and so may have damaged them. The third harvest was delayed by heavy rainfall over the autumn and winter and was done in March 2011. The yield was poor at 19.1 t ha^{-1}. The harvest was followed promptly by applications of Fibrophos™, at a rate of 660 kg ha^{-1}, and a total of 20 tonnes of lime. 200 tonnes of wood-waste compost was applied on 31 March 2011. The crop was next harvested on 31 October 2013.

Sub-site C has been in continuous use for arable crops with a rotation, typical of the area, of 2-3 years of winter wheat followed by oil seed rape (OSR) as a break crop. A second consecutive winter wheat crop was planted on the 30 September 2011. Nitrogen fertilisers were applied on four dates in 2012: 2 March, 22 March, 4 May and 21 May at rates of 40, 41, 77 and 20 kg ha^{-1} respectively. On 22nd March sulphur was also applied at a rate 37 kg ha^{-1}. The crop was harvested on 7th September 2012. Prolonged heavy rainfall throughout the winter of 2012/13 resulted in the farmer being unable to drill any crops over this period and the soil conditions in the measurement field were particularly bad, so no crop was planted that spring. As a result, it was decided to transfer the measurements to another field, close by, which had an identical land management history. Spring barley was planted in this field on 7th April 2013. Fertilisers were applied on 26 April, 20 May and 4 June. The rate of application of N was 50, 70 and 43 kg ha^{-1} respectively. The fertiliser applied on 26 April also had P, K and SO_3 at rates of 20, 30 and 19 kg ha^{-1}. The crop was harvested on 29 August and OSR was planted on 4 September 2013.

Meteorological conditions at the site, during the period of measurements, are illustrated in Figure 2.5. During 2012, monthly rainfall totals higher than the long-term averages were recorded in seven months. Large totals were recorded in May and July; 2.8 and 2.4 times

the long-term average respectively. In contrast, during 2013, the monthly rainfall totals were less than the long-term averages in all months except March, June and October. The rainfall total for October was nearly twice the long-term average. Over most of the period, the monthly average air temperatures were fairly close to the long-term averages. Exceptions to this occurred in the winter of 2011/12, when the monthly averages were several degrees below the long-term averages, and for the first three months of 2013, when the monthly average air temperatures were again below the long-term averages.

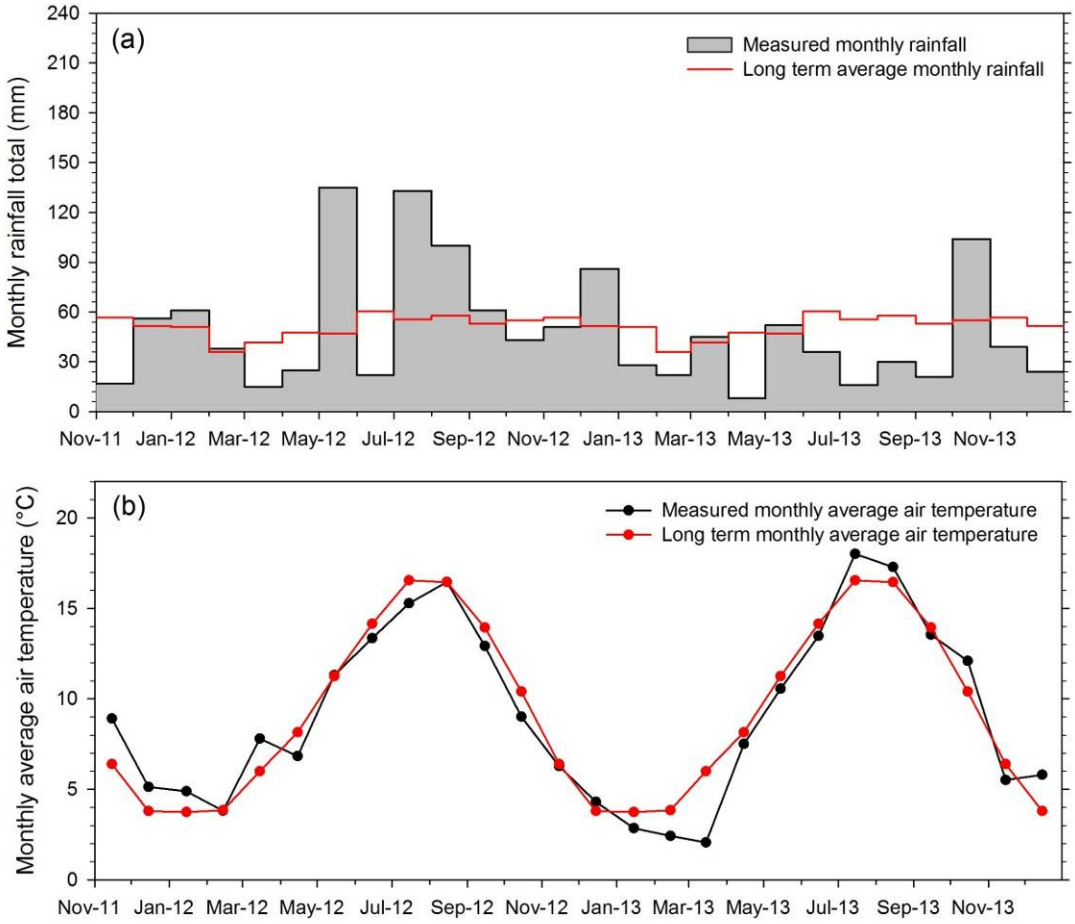

Figure 2.5: Measured monthly rainfall totals (a) and monthly average air temperature (b) compared to the long-term (1981-2010) averages for the Lincolnshire field site.

2.3 West Sussex

Sub-site A was under set-aside from 2000 to 2004 with the vegetation cut in July or August of each year. Only the headlands were cut in 2005. In preparation for planting the SRC willow, the field was ploughed in 2007 which was followed by the application of Glyphos Supreme 3.5 l ha^{-1} and Dursban 1 kg ha^{-1} in October. The field was power harrowed in April 2008 followed by the application of Glyphos Supreme 3.5 l ha^{-1} to green parts of the field in June. The SRC willow was planted, at 15,000 cuttings ha^{-1}. Prior to emergence, Flexidor 2 l ha^{-1}, Stomp 3.3 l ha^{-1} and Dursban 1 kg ha^{-1} were applied. The willow was cut back in March 2009 and Weedazol was applied at 10 l ha^{-1}.

Sub-site B was under set-aside from 2000 to 2004. In 2005 it was entered into the Entry Level Stewardship (ELS) scheme as permanent grass with low inputs. The ELS expired in November 2010 and was renewed in 2011. Management of the grass consists of one week of grazing by sheep, approximately twice a year.

Meteorological conditions at the site during the period of measurements are illustrated in Figure 2.6. During 2012, the summer months were particularly wet with monthly rainfall totals above the long-term averages from May until November inclusive. Large totals were recorded in April and June and October; 4.1, 5.9 and 1.7 times the long-term averages respectively. In contrast, during 2013, the monthly rainfall totals were less than the long-term averages in all months except March, October and December, the latter two being 1.5 and 1.6 times the long-term average respectively. Over most of the period, the monthly average air temperatures were fairly close to the long-term averages. Exceptions to this occurred in the winter of 2011/12, when the monthly averages were several degrees below the long-term averages, and for the first five months of 2013, when the monthly average air temperatures were again below the long-term averages.

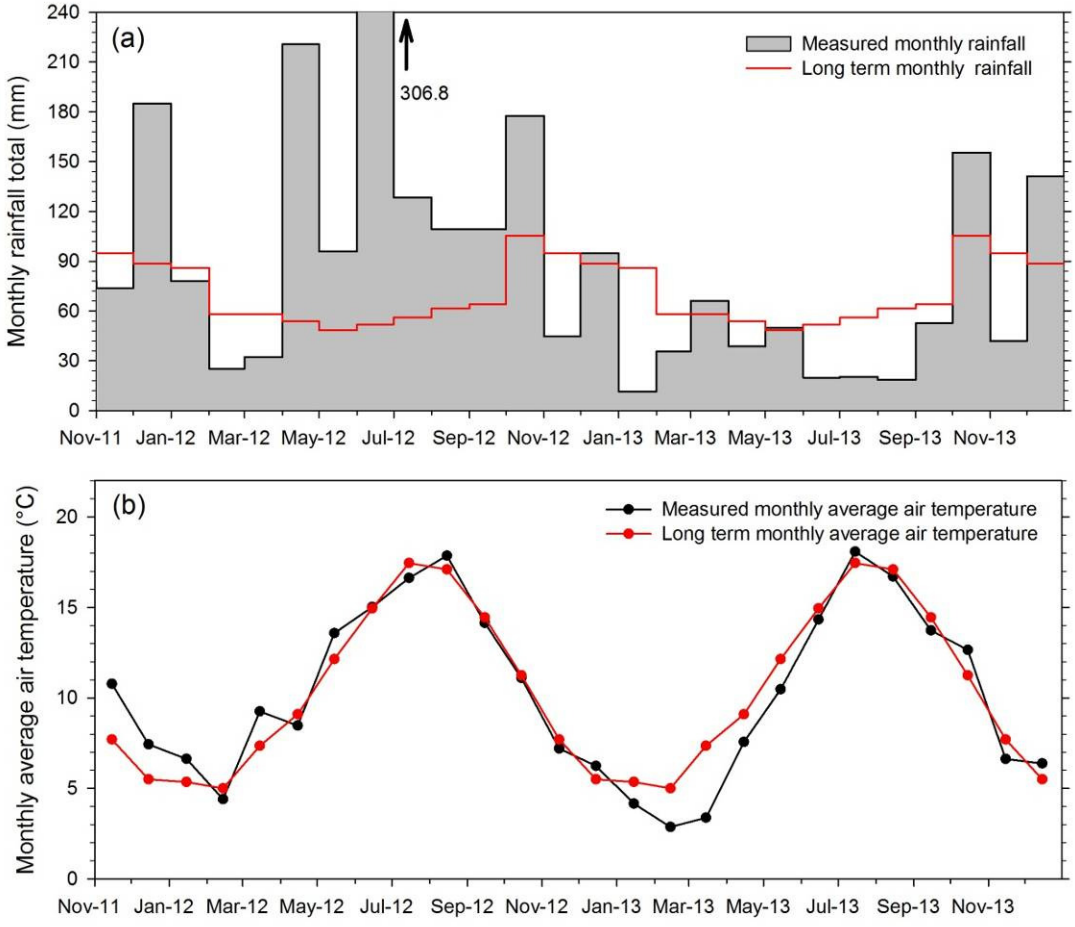

Figure 2.6: Measured monthly rainfall totals (a) and monthly average air temperature (b) compared to the long-term (1981-2010) averages for the West Sussex field site.

3. WHOLE SYSTEM CARBON FLUXES

Summary

1. EC measurements were made at all network sites: Aberystwyth: *Miscanthus x giganteus*, East Grange: SRF, Lincolnshire: *Miscanthus x giganteus*, SRC willow and arable, West Sussex: SRC willow and grassland.

2. These data were processed, quality controlled, gap-filled and the NEE partitioned into TER and GPP.

3. The two land covers which have positive values for the NEE, i.e. sources of carbon, are the grass at the West Sussex site and the land-cover conversion to *Miscanthus x giganteus* at Aberystwyth sub-site A.

4. At Aberystwyth sub-site A, the result for the *Miscanthus x giganteus*, being a source of carbon in the transition year, 2012, is not surprising but, in the following year, it had become a sink which suggests that, in terms of the carbon balance, the carbon debt of transition is likely to be repaid relatively quickly.

5. The annual GPP for the two *Miscanthus x giganteus* crops are fairly similar despite these being second year (Aberystwyth) and sixth year (Lincolnshire). The annual TER at the sites show a big difference, that at the Lincolnshire site is about 60% of that at the Aberystwyth site. A reasonable explanation for this difference is that the disturbance of the land-cover change at Aberystwyth is still affecting the respiration.

6. The annual TER for the SRC willow at the Lincolnshire and West Sussex sites are similar, but the annual GPP at the West Sussex site is about 27% higher than that at the Lincolnshire site.

7. The greatest carbon sink is the SRF at the East Grange site, followed by the SRC willow at the West Sussex site. The third greatest carbon sink is the winter wheat at the Lincolnshire, but the figure for this land cover is an under-estimate as the measurements did not cover the full period of this crop.

8. The period of marked GPP rates for the SRC willow and the *Miscanthus x giganteus* have about the same length, roughly six months, but the SRC willow occurs about a month earlier than the *Miscanthus x giganteus*.

3.1 Methods and Materials

The net ecosystem exchange (NEE) in croplands and forests is determined by the difference between CO_2 uptake, through photosynthesis, and CO_2 loss, through plant and soil respiration (negative values indicate take up, by the land surface from the atmosphere). EC is a technique that is widely applied to measure this at the ecosystem level. Consequently, this project made use of existing systems and supplemented these by wholly-funded (West Sussex and an additional system at Aberystwyth sub-site A) and partially-funded

(Lincolnshire sub-site C) systems. All the EC systems used were set to 20 Hz sampling to produce 30-minute average fluxes. Ancillary measurements were all set to be logged as 30-minute averages, except in the case of rainfall, which was logged as 30-minute totals. Detailed descriptions of the systems can be found below. Table 3.1 is a summary of the main instruments, that are directly relevant to the eddy covariance, at each sub-site.

Table 3.1: Summary of the instruments, directly relevant to EC, at each sub-site

Network site	Sub-site	IRGA	Sonic anemometer	Logger	Net radiometer	Soil heat flux plates
Aberystwyth	A*	Campbell Scientific EC150	Campbell Scientific CSAT3	Campbell Scientific CR3000	Kipp & Zonen NR Lite	Hukseflux HFP01SC
East Grange	A	Campbell Scientific EC150	Campbell Scientific CSAT3**	Campbell Scientific CR3000	Kipp & Zonen NR Lite	Hukseflux HFP01SC
Lincolnshire	A	LI-COR LI-7500A	Gill R3	IH Hydra Mk4	Kipp & Zonen CNR 1	Hukseflux HFP01SC
	B	LI-COR LI-7500A	Gill R3	IH Hydra Mk4	Kipp & Zonen CNR 1	Hukseflux HFP01SC
	C	LI-COR LI-7500A	Gill R3	Campbell Scientific CR3000	Hukseflux NR01	Hukseflux HFP01SC
West Sussex	A	LI-COR LI-7500A	Gill Windmaster	LI-COR LI-7550	Kipp & Zonen NR Lite	Hukseflux HFP01SC
	B	LI-COR LI-7500A	Gill Windmaster	LI-COR LI-7550	Kipp & Zonen NR Lite	Hukseflux HFP01SC

* Two identical systems were deployed in this sub-site

** The path between the sensors was enclosed by a custom made jacket from November 2012 on

3.1.1 Aberystwyth

Two identical EC systems were deployed in the sub-site A, the conversion to *Miscanthus x giganteus*. This was done in order to reduce the number of gaps in the time series of NEE that would have occurred with a single EC system as a result of the geometry of the field, in that it is surrounded by woodlands, and the variability in the wind direction associated with diurnal coastal winds. The EC systems were supplied by Campbell Scientific as EC150 open-path eddy covariance systems; these consisted of the CSAT3 sonic anemometer with the EC150 Open-Path infra-red gas analyser (IRGA) and an HMP155 air temperature and relative humidity probe. Initial sensor management was by an EC100 control box through to a CR3000 datalogger. This system was complemented with ground energy balance sensors; two CS616 water reflectometers, two TCAV averaging soil temperature thermocouples and two HFP01SC soil heat flux sensors. The first EC system was deployed on 3rd January 2012. It was removed on 4th April 2012, immediately prior to ploughing and re-installed immediately following planting of the Miscanthus x giganteus on 24th Apr 2012. The second EC system was installed on 9th January 2013

The meteorological station carried the following sensors: Young's 52203 tipping bucket rain-gauge; Young's 5103 wind monitor; NR Lite net radiometer; Skye Instruments SKP215 quantum radiation sensor; three CS616 soil water reflectometers at two depths; three TCAV averaging soil temperature sesnors at two depths; two HFP01SC soil heat flux plates. Data from these sensors were collated through a CR1000 datalogger.

3.1.2 East Grange

The EC system over the SRF was supplied by Campbell Scientific Ltd. and consisted of a CSAT3 Sonic anemometer and a CS7500 Open Path IRGA, logged to a CR3000. The IRGA was modified by enclosing the open-path sensor within a jacket so as to produce a hybrid for the second reporting year, 2013. The meteorological station consisted of six CS616 TDR 30 cm probes inserted vertically; a HFP01SC soil heat flux plate at a depth of 5 cm; a CS300 pyranometer; a NR-LITE net radiometer; an ARG100 RainGauge; a HMP45C air temperature and relative humidity sensor, all logged with a CR1000 data logger.

3.1.3 Lincolnshire

The three EC systems used LI-COR LI-7500A open-path IRGAs and Gill R3 sonic anemometers. At sub-sites A and B the data was logged with IH Mk4 Hydra data loggers whilst that at sub-site C was logged with a Campbell Scientific CR3000 logger. At each of the three sites, there were two HFP01SC soil heat flux plates at 5 cm and two PT107 soil temperature probes at depths of 2.5 cm. At each of sub-sites A and B the soil water content at 2.5 cm was measured using two CS616 TDR 30 cm probes and the net radiation and its components are measured using a CNR1 net radiometer. At sub-site C these measurements were provided by two DeltaT SM200 soil moisture sensors and a Campbell Scientific NR01-L net radiometer. These data were logged, as 30-minute averages, using two CR10s at sub-site A, a CR10x at sub-site B and the CR3000 at sub-site C.

Meteorological data were provided by a Didcot Instruments AWS with: cup anemometer, wind vane, air and wet bulb temperatures and a raingauge (Rimco) located at sub-site A. A second raingauge was at sub-site B. These data were logged as 30-minute averages, except the rainfall which was the 30-minute total, on a CR10 logger. Two DeltaT ProfileProbes provided measurements of soil water contents at sub-site A, down to a depth of 1 m; these data were logged as 30-minute spot readings on a CR10 logger. Soil water contents were measured at depths of 20 and 30 cm with DeltaT SM200 soil moisture sensors at sub-site C.

3.1.4 West Sussex

The two identical EC systems, over the SRC willow and the grass, used LI-COR LI-7500A open-path IRGAs and Gill Windmaster sonic anemometers. These connected directly to LI-COR LI-7550 Analyzer interface units where data were logged onto an industrial-grade USB stick.

Additional measurements included four HFP01SC soil heat flux plates, two TCAV soil temperature probes, two CS616 TDR 30 cm probes to measure soil water content, one

NR-LITE net radiometer, one SKP215 quantum sensor, one HMP155A air temperature and relative humidity probe, one Young's 52203 rain gauge and a Young's 05103-5 wind monitor. All data were logged to a CR1000 data logger.

3.1.5 EC data processing and QC

The basis of the processing and quality control (QC) procedures used was to follow the CarboEurope procedures which are, in turn, based on the EUROFLUX procedures (Aubinet, 1999). The 20 Hz raw data were processed to produce 30-minute average values of the CO_2 (NEE) fluxes using the EddyPro software (LI-COR Biosciences). Further QC procedures used included spike identification and removal (Papale *et al.* 2006) and U* filtering to identify periods when bias and uncertainties can arise due to insufficient turbulent mixing, which the EC method relies on. Finally a footprint analysis was carried out using the method of Neftel *et al.* (2008). Data were rejected when, over a 30-minute period, the signal from the land cover of interest was less than 70% of the total signal. The exception to this footprint analysis were the data from the West Sussex site where the wind direction measurements were used to reject data when the wind direction was not between 135° and 262° for the SRC willow, and not between 140° and 290° for the grass.

The missing values in the 30-minute data were gap-filled using procedures made available by the Department of Biogeochemical Integration at the Max Planck Institute for Biogeochemistry at http://www.bgc-jena.mpg.de/~MDIwork/eddyproc/. The procedures were similar to those described by Falge *et al.* (2001), but considered both the co-variation of fluxes with meteorological variables and the temporal auto-correlation of the fluxes (Reichstein *et al*. 2005), whereby the missing value is replaced by the average value under similar meteorological conditions.

Flux partitioning of the NEE, into gross primary productivity (GPP) and total ecosystem respiration (TER), was performed using procedures made available by the Department of Biogeochemical Integration. These make use of the procedure of Lloyd and Taylor (1994) to estimate respiration as a function of temperature. The parameters of the Lloyd and Taylor (1994) model are calibrated, using the measured night-time NEE data for a given time period. This model is then used to estimate the TER for each 30-minute value. The estimated TER is then subtracted from the NEE to estimate the GPP. It should be noted that GPP is the amount of chemical energy, as biomass, that primary producers create in a given length of time, i.e. it is not a flux. Consequently, it is quite common in the literature not to be specified as negative. In this report the convention of not using a negative sign has been used to improve clarity, particularly in the graphs.

3.2 Results

3.2.1 Aberystwyth

For the first three months of 2012, the daily average NEE measured from sub-site A (Miscanthus x giganteus) was generally positive and in the range of 0 to 80 mg CO_2-C m^{-2} h^{-1}, Figure 3.1. It was typically a balance of roughly equal GPP and TER fluxes. Following conversion in March 2012, the GPP was consistently low, averaging 31 mg CO_2-C m^{-2} h^{-1} over the next six weeks whilst the NEE was dominated by TER. The GPP began to

increase in early June and was generally in balance with the TER so that the NEE remained around zero. The situation changed around mid-August when the GPP began to exceed the TER, resulting in a generally negative NEE, i.e. a period of net gain in carbon by the land surface. This continued until early November, albeit with the TER and GPP declining with the reduction in downward global solar radiation and air temperature. As the *Miscanthus x giganteus* senesced over the next four to five weeks, the GPP diminished significantly so that the NEE was dominated by the TER. This remained the situation through the winter. The GPP showed a slight increase in mid-February 2013, to an average of 75 mg CO_2-C m^{-2} h^{-1}, which continued until early June. This resulted in the NEE generally being negative until mid-April when, following the application of herbicides, the TER increased, resulting in a generally positive NEE. The emergence of the new shoots in early June resulted in an increase in GPP so that NEE returned to being about zero for several weeks. However, from the end of June onwards, the GPP exceeded the TER resulting in the NEE generally being negative. i.e. a net gain of carbon by the land surface, until the end of September. By the end of November, the GPP had declined to close to zero with the result that the NEE was dominated by the TER, i.e. a net loss of carbon to the atmosphere.

For the 12 months of 2012, the total carbon lost through respiration was 1125 g CO_2-C m^{-2} whilst the total carbon gained was 861 g CO_2-C m^{-2}, i.e. a net loss of 264 g CO_2-C m^{-2}. For 2013 the figures are a total loss of 1373 g CO_2-C m^{-2} whilst the total carbon gained was 1493 g CO_2-C m^{-2}, i.e. a net gain of 120 g CO_2-C m^{-2}. Thus, over these two years, the net loss of carbon was of 144 g CO_2-C m^{-2}.

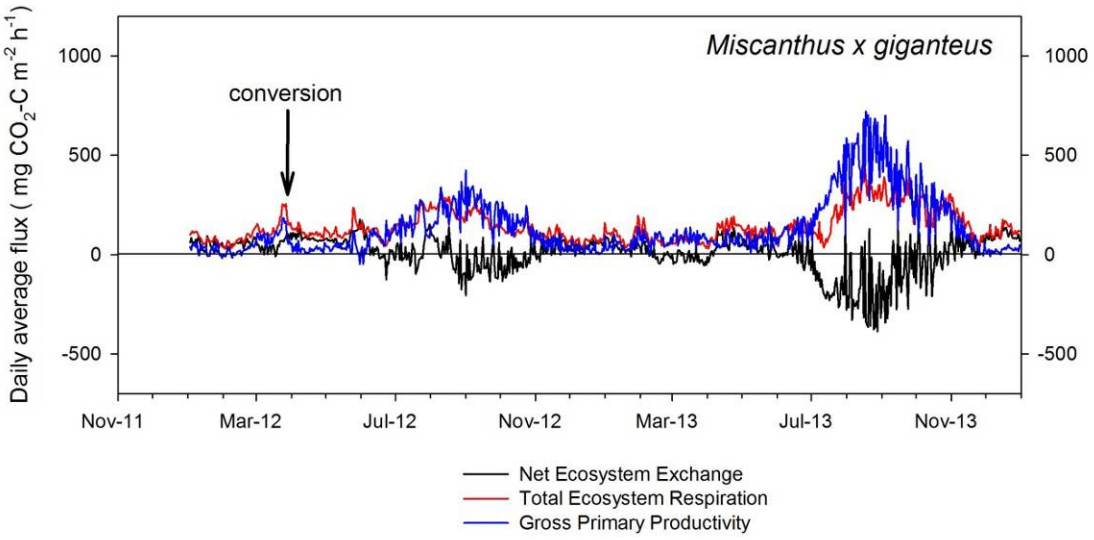

Figure 3.1: Measured and gap-filled daily average NEE and GPP and TER, derived from the NEE, by flux partitioning for Aberystwyth sub-site A (*Miscanthus x giganteus*).

3.2.2 East Grange

During 2012 the daily average NEE values (SRF) were invariably negative, implying an excess of GPP over TER even over the winter months, (Figure 3.2). GPP rates began to rise in mid-February until the second half of April, after which they fluctuated around a plateau of about 275 mg CO_2-C m^{-2} h^{-1}. The values of TER show a similar trend but remain well below those of GPP, except during a couple of weeks in mid-September. By the end of November

the NEE values had declined to levels recorded early in the year. The daily average NEE values recorded during 2013 are well below those of 2012, which is surprising as the meteorological conditions were more favourable, e.g. air temperatures were higher during the main growing season, see Figure 2.3. Several potential causes of this are currently under investigation so no analysis of these data will be attempted at this stage.

For the 12 months of 2012, the total carbon lost through respiration was 403 g CO_2-C m^{-2} whilst the total carbon gained was 1447 g CO_2-C m^{-2}, i.e. a net gain of 1044 g CO_2-C m^{-2}, i.e. a large gain.

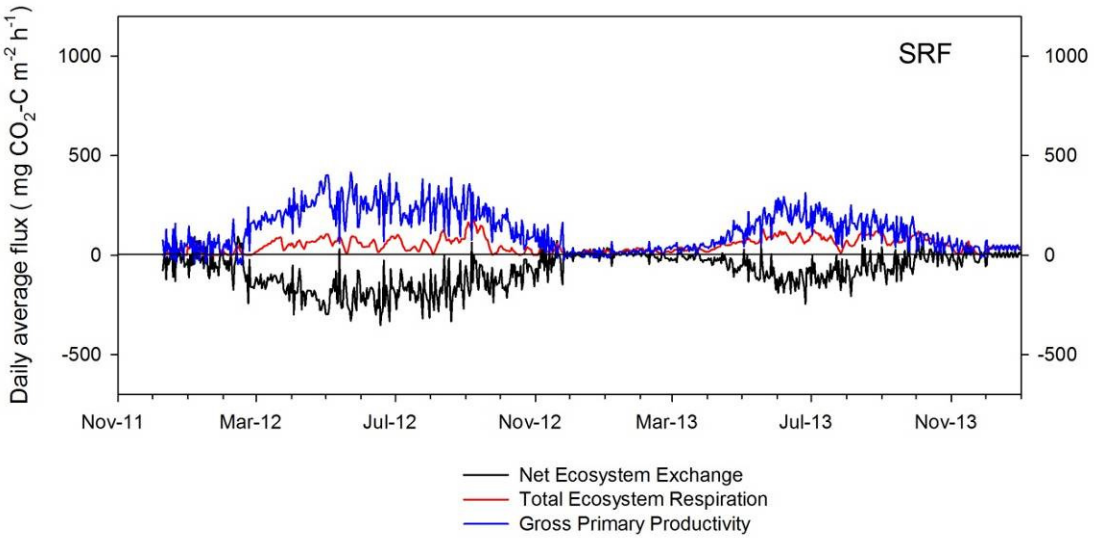

Figure 3.2: Measured and gap filled daily average NEE and GPP and TER, derived from the NEE, by flux partitioning for East Grange sub-site A (short rotation forestry).

3.2.3 Lincolnshire

3.2.3.1 Miscanthus x giganteus

During December 2011 and January 2012, the measured daily average NEE of the *Miscanthus x giganteus* was generally between 30 and 60 mg CO_2-C m^{-2} h^{-1} (Figure 3.3 a), with the TER being very much the dominant component. Following the harvest, the NEE remained low until early June, when the flux began to increase, following the appearance of shoots in early May. The highest rates of NEE occurred between late July and early September, during which period the GPP was about twice the TER. The NEE rates then decreased until the middle of November, by which date the NEE rates were again in the range of 30 to 60 mg CO_2-C m^{-2} h^{-1}. Senescence began in the crop around the middle of October and was complete by the end of November. There is a large gap in the measurements between 10th March and 4th July 2013 due to the necessity of removing the instruments for the harvesting and baling of the crop; this was then followed by harrowing - to spread the rhizomes - and the application of wood waste. From when the measurements resumed and until the end of 2013, the NEE values were usually low as the TER and GPP were generally balancing each other, except during August when the GPP was persistently greater than the TER. The TER rates observed were much greater than those observed during the same period in 2012, indicating that the harrowing had resulted in an increased release of carbon.

For the 12 months of 2012, the total carbon lost through respiration was 781 g CO_2-C m^{-2} whilst the total carbon gained was 1264 g CO_2-C m^{-2}, i.e. a net gain of 483 g CO_2-C m^{-2}. These figures are an under-estimate, due to the loss of data for two and a half months but this was a period of low fluxes and so the measured totals are probably only slightly less than would have been recorded if measurements had been for the full year.

3.2.3.2 SRC willow

The NEE rates measured from the SRC willow, in the first three months of 2012, were generally close to zero (Figure 3.3 b), with the TER generally balanced by the GPP, the latter coming presumably from a sparse understory of brambles and broadleaf weeds. The NEE fluxes began to grow from early March, coinciding with budburst of the crop and levelled out to fluctuations around a plateau of around 380 mg CO_2-C m^{-2} h^{-1} from early June to late July, before declining again until mid-November, after which the NEE rates were generally constant through the winter months. Unfortunately an intermittent fault in the EC IRGA meant that little data was collected during the main growing period of 2013 so there is little to comment on before the crop was harvested early in October 2013, except that the rise in NEE rates in spring began about a month later than in 2012 as a consequence of the colder weather. For the 12 months of 2012, the total carbon lost through respiration was 1042 g CO_2-C m^{-2} whilst the total carbon gained was 1475 g CO_2-C m^{-2}, i.e. a net gain of 432 g CO_2-C m^{-2}.

3.2.3.3 Arable

The NEE rates measured over winter wheat were the highest recorded at this field site, Figure 3.3c. GPP was already high when the measurements began, in early April 2012, and continued to increase to a peak during the last week of May before declining as the crop ripened. In mid-July the NEE rates changed from negative to positive values; indicating that the TER rates were greater than the GPP rates. The crop was ready for harvest by early August. Over the 125 days of measurements, the total carbon lost through respiration was 1056 g CO_2-C m^{-2} whilst the total carbon gained was 1721 g CO_2-C m^{-2}, i.e. a net gain of 665 g CO_2-C m^{-2}. In 2013, measurements began soon after spring barley had been drilled. In early June, the NEE rates were increasing to be at their highest in the first two weeks of July as a result of high GPP rates. Following this period, both TER and GPP rates declined and NEE rates were around zero at the start of August. Over the 98 days of measurements, the total carbon lost through respiration was 607 g CO_2-C m^{-2} whilst the total carbon gained was 854 g CO_2-C m^{-2}, i.e. a net gain of 247 g CO_2-C m^{-2}. Following the planting of a crop of OSR early in September, the NEE rates continued close to zero although this was due to the TER and GPP rates essentially balancing each other.

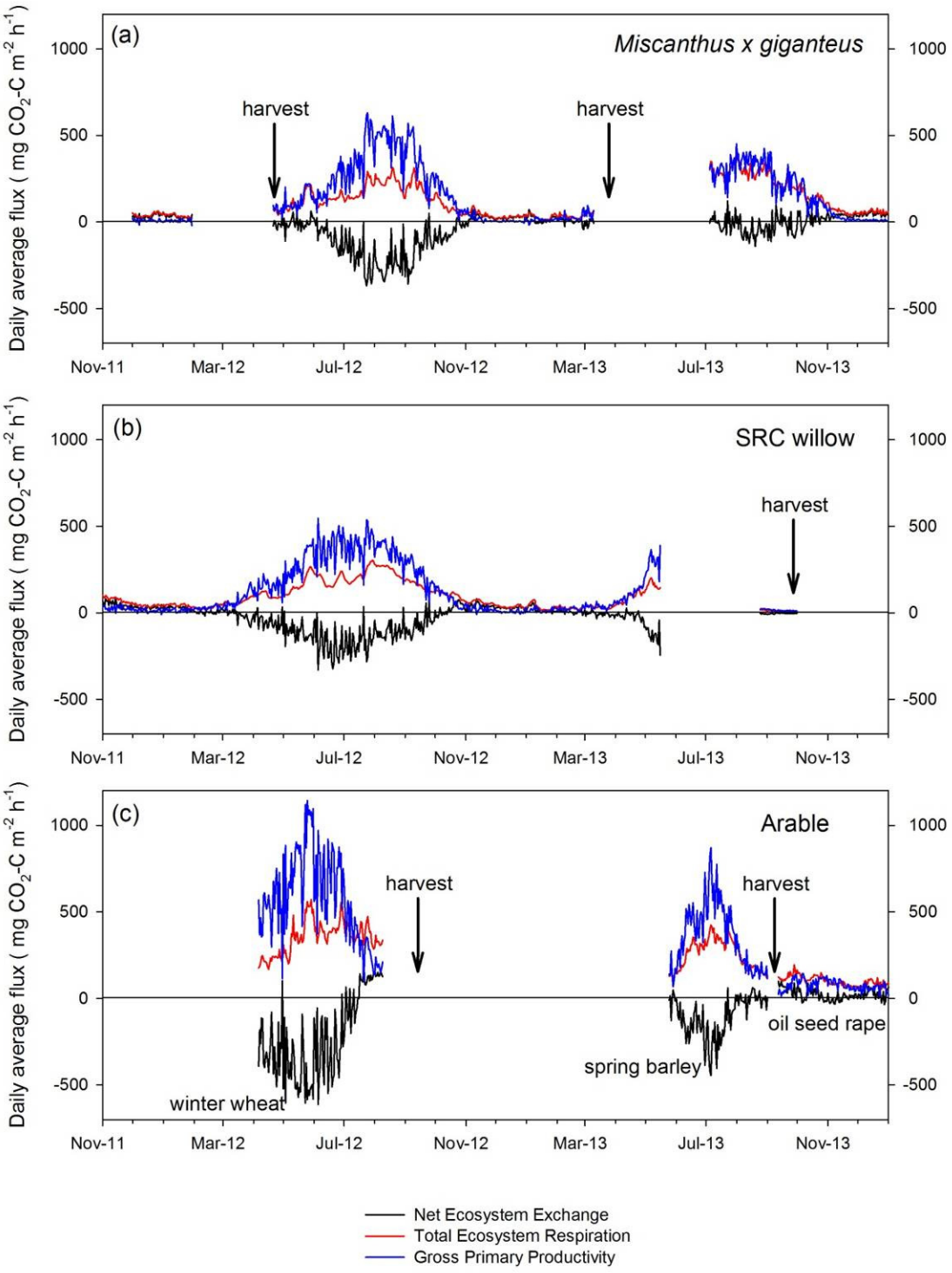

Figure 3.3: Measured and gap-filled daily average NEE and GPP and TER, derived from the NEE, by flux partitioning for the Lincolnshire site (a) – sub-site A, *Miscanthus x giganteus* (b) – sub-site B, short rotation coppice willow (c) – sub-site C, arable.

3.2.4 West Sussex

3.2.4.1 SRC willow

Generally, the NEE rates recorded from the SRC willow averaged about zero from the start of 2013 until mid-April, representing a balance between TER and GPP (Figure 3.4a). Following budburst in April, the NEE is negative until mid-October, representing a dominance of GPP over TER with the highest values of GPP being recorded from the start of June to the end of July. Over the same two months there is a trend of increasing TER. At the end of July there is a rapid drop in the values of GPP and TER. There is no obvious reason for this although, given that the rainfall is particularly low and follows the first six months of the year having a rainfall total that is below the long-term average (Figure 2.6), it is possible that stress due to low soil water availability may be responsible. The last two months of the year tend to show TER dominating over GPP. Over the twelve months of 2013, the total carbon lost through respiration was 990 g CO_2-C m^{-2} whilst the total carbon gained was 1872 g CO_2-C m^{-2}, i.e. a net gain of 882 g CO_2-C m^{-2}.

3.2.4.2 Grassland

At the grass sub-site at the end of 2012, the TER rates dominate the NEE with the GPP rates being around the zero line (Figure 3.4 b). From the start of 2013 until mid-July, the TER and GPP rates balance each other out, shown by the NEE rates being around zero. From mid-July until the end of September the NEE is consistently positive, indicating that the TER exceeds the GPP. There is no obvious reason for this in the data but it could again be due to low soil water availbility. From late September through to early November the NEE rates change to negative values which is more a function of a reduction in TER rather than an increase in GPP. For the remainder of the year the NEE values return to being positive, indicating a dominance of TER over GPP. Over the twelve months of 2013, the total carbon lost through respiration was 1302 g CO_2-C m^{-2} whilst the total carbon gained was 1067 g CO_2-C m^{-2}, i.e. a net loss of 235 g CO_2-C m^{-2}.

Comparing the carbon fluxes for the two land covers for 2013, the annual totals for the TER and the GPP for the SRC willow were higher than those for the grass. More importantly, the grass was a net source of CO_2 whilst the SRC willow was a net sink.

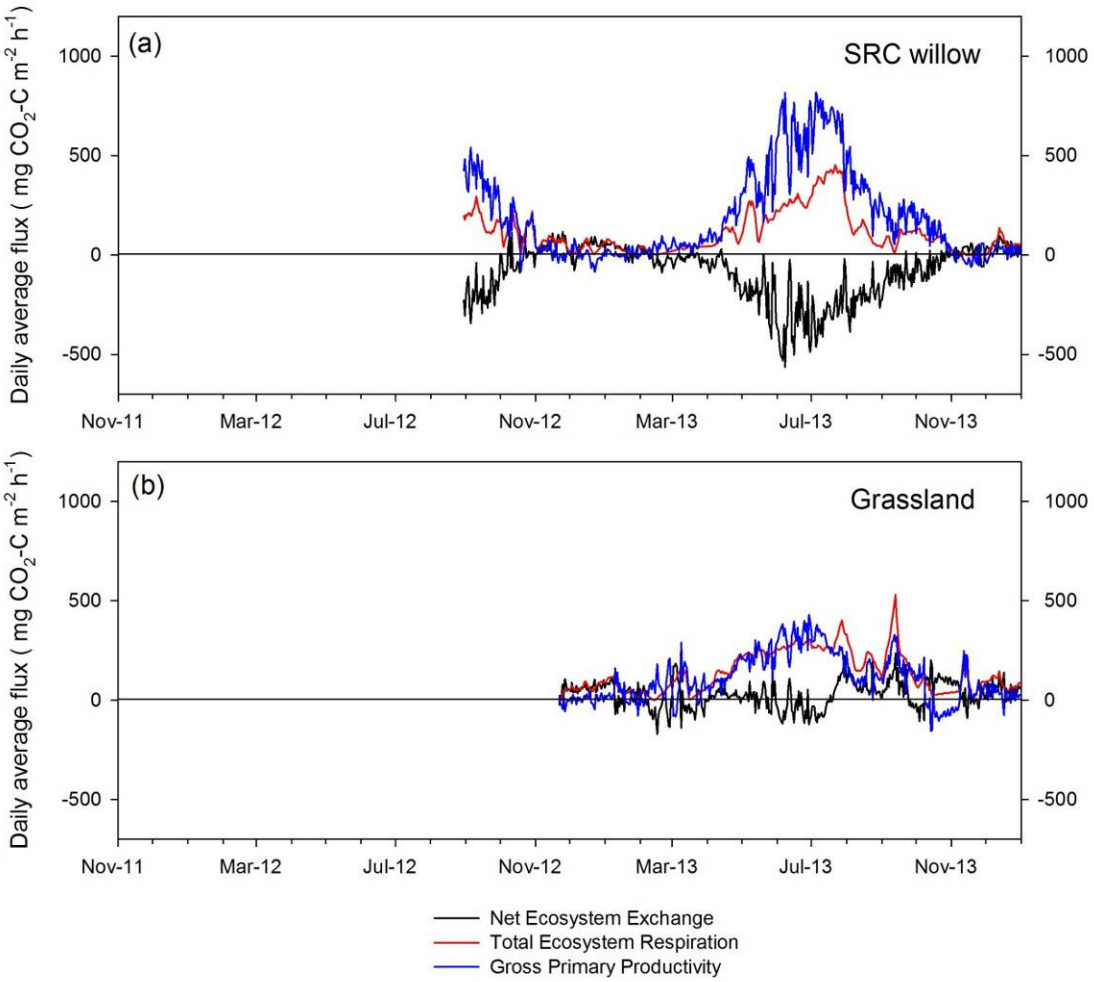

Figure 3.4: Measured and gap-filled daily average NEE and GPP and TER, derived from the NEE, by flux partitioning for the West Sussex site (a) – sub-site A, short rotation coppice willow (b) – sub-site B, grassland.

3.3 Discussion

Although differences in the climate and soils make meaningful comparisons between different field sites difficult, it is possible to make a number of comments about the major differences between the land covers; this is in terms of both the annual carbon balances of the perennial land covers and the carbon balances over the lifetime of the arable crops. The values for these are given in Table 3.2.

The two land covers which have positive values for the NEE, i.e. sources of carbon, are the grass at the West Sussex site and the land-cover conversion to *Miscanthus x giganteus*, in 2012, at the Aberystwyth site. It is not possible to generalise about grass from this one result at the West Sussex field site as grass can be either a sink or a source of carbon (see Gilamanov *et al.*, 2007). The result for the *Miscanthus x giganteus* in the transition year, 2012, is not surprising but, in the following year, it had become a sink which suggests that, in terms of the carbon balance, the carbon debt of transition is likely to be repaid relatively quickly.

Table 3.2: Summary of the whole system carbon balances of land cover types at the field sites

Field site	Land cover	TER (g CO_2-C m^{-2})	GPP (g CO_2-C m^{-2})	NEE* (g CO_2-C m^{-2})
Aberystwyth	*Miscanthus x giganteus* 2012	1125	861	264
	Miscanthus x giganteus 2013	1373	1493	-120
East Grange	SRF 2012	403	1447	-1044
Lincolnshire	*Miscanthus x giganteus* 2012	781	1264	-483
	SRC willow 2012	1042	1475	-433
	winter wheat**	1056	1721	-665
	spring barley***	607	854	-247
West Sussex	SRC willow 2013	990	1872	-882
	grass 2013	1302	1067	235

* negative values of NEE denote carbon sequestration whilst positive denote carbon emissions
** 5 April – 8 August 2012
*** 25 May – 1 September 2013

The annual GPP for the two *Miscanthus x giganteus* crops are fairly similar despite these being second year (Aberystwyth) and sixth year (Lincolnshire) crops. A possible explanation might be a lower nutrient availability in the soil at the Lincolnshire site. This is suggested by the higher total %N found in the soils at Aberystwyth sub-site A compared to the values found in the soils at the Lincolnshire sub-site A (see Table 4.1 below), but more detailed measurements are required to confirm this. The annual TER at the sites do show a big difference, that at the Lincolnshire site is about 60% of that at the Aberystwyth site. A reasonable explanation for this difference is that the disturbance of the land-cover change at Aberystwyth is still affecting the respiration.

In comparison, the annual TER for the SRC willow at the Lincolnshire and West Sussex sites are similar, but the annual GPP at the West Sussex site is about 27% higher than that at the Lincolnshire site. Explanations for this might be that the SRC willow at the West Sussex site was in year four of a rotation whilst that at Lincolnshire was in year two. Alternately a lower fertility of the soil at the Lincolnshire site might be responsible.

The greatest carbon sink is the SRF at the East Grange site, followed by the SRC willow at the West Sussex site. However, the figures for the SRF should be treated with caution because the trees are still very immature and so the grass substrate may be contributing a sizeable proportion to the NEE. The third greatest carbon sink is the winter wheat at the Lincolnshire site, but the figure for this land cover is an under-estimate as the measurements did not cover the full period of this crop.

The period of marked GPP rates for the SRC willow and the *Miscanthus x giganteus* have about the same length, roughly six months, but the SRC willow occurs about a month earlier than the *Miscanthus x giganteus*.

4. SOIL CARBON STOCKS AND GHG EMISSION

Summary

1. Land-use change to bioenergy crops is likely to influence soil microbial activity and associated fluxes of greenhouse gases (GHGs) from soils. Uncertainty in soil greenhouse gas (GHG) fluxes across different land-use transitions is an issue that needs to be addressed for the development of more accurate life-cycle analyses (LCA's) for bioenergy crops.

2. The ELUM Network sites were established to examine different land-use transitions covering arable to bioenergy and grass to bioenergy. At these sites fluxes of soil CO_2, CH_4 and N_2O were measured monthly over a two year period, along with environmental measurements, including air/soil temperature, soil moisture and litter fall.

3. Significant reductions in N_2O emissions, following a switch from arable crop to woody, perennial bioenergy crops were observed. For the Lincolnshire arable sub-site average N_2O emissions were 8.03 and 24.1 mg CO_2 eq h^{-1} in 2012 and 2013 respectively whilst in the bioenergy crops present at this site average N_2O emissions ranged from 0.02-1.70 mg CO_2 eq h^{-1}. Similar trends were observed with the arable sub-site at East Grange versus the bioenergy crops. This is most likely linked to reductions in fertiliser application following this transition and therefore management of the bioenergy crop will be important in determining whether valuable reductions in N_2O are maintained.

4. Across all bioenergy land-uses fluxes of CH_4 and N_2O were shown to be close to negligible. Average CH_4 fluxes ranged from low levels of oxidation (-0.39 mg CO_2 eq h^{-1}) to very low levels of CH_4 production (+0.05 mg CO_2 eq h^{-1}). Average N_2O emissions ranged from 0.02-3.86 mg CO_2 eq h^{-1} across the bioenergy sites with no significant differences between bioenergy and reference crop at the sites. High emissions of CH_4 and N_2O were observed at the Aberystwyth conversion site but these were observed in both the reference site and the *Miscanthus x giganteus* site. These higher fluxes showed high within-site variability and did not appear to be related to the planting of the bioenergy crop.

5. Fluxes of CO_2 were partitioned in to hetero- and autotrophic sources in order to obtain an estimate of microbial respiration rates. In general heterotrophic respiration from soils under bioenergy was lower, which suggests that microbial turnover of C is reduced in these bioenergy systems. However, it is important to note that there is much uncertainty regarding source partitioning and we recommend that research is carried out in order in order to provide more accurate comparisons of heterotrophic respiration between different crops.

4.1. Introduction

Land-use change to bioenergy crops is likely to affect soil cycling of C and N, with potential benefits for C sequestration under perennial bioenergy crops. This can result directly from the crop planted with changes in C input, either through litter (Stauffer *et al.*, 2014) or from root exudates (Anderson-Teixeira *et al.*, 2013), being shown to directly affect microbial activity. In addition the conversion of arable to perennial bioenergy crops results in changes to the management regime, including potential reductions in fertiliser application (Kavdir *et*

al., 2008) and soil tillage. Reductions in these management processes have been shown to result in significant reductions in the fluxes of CH_4 and N_2O (Hansen *et al.*, 1993), which have global warming potentials (GWPs) of 23 and 296 times that of CO_2 respectively (IPCC 2001). Uncertainty in soil GHG fluxes across different land-use transitions is an issue that needs to be addressed for the development of more accurate LCAs for bioenergy crops (Hillier *et al.*, 2009).

Microbial activity is driven by environmental factors and will respond to changes in temperature and soil moisture resulting from seasonal variation throughout the year. Monitoring of GHG emissions throughout these seasonal variations is essential for establishing if and how different cropped soils interact with environmental factors. This informs models of soil GHG dynamics with regard to how microbial activity responds to land-use change both under current climate conditions and with future climate change scenarios.

As discussed in Section 3 of this report, eddy covariance (EC) provides valuable information regarding net ecosystem exchange (NEE) and the relative proportions of gross primary productivity (GPP) and ecosystem respiration. The value of the chamber technique, employed in this Section, is that it allows the determination of non-CO_2 GHG and, in most cases, the soil component of the CO_2 emissions. In this section we provide an overview of the GHG data fed into the LUC and crop management model (deliverable D4.3 - BI1001_PM07.4.3_WP4_LUC and Crop Management Model v1.0) and summarise the findings from the four network sites.

4.2. Methods

4.2.1 Soil GHG measurement across the network sites

Soil GHG fluxes were measured on a monthly basis from each of the network sites using the protocols outlined in Appendix 1 of Deliverable D3.2 (BI1001_PM04.3.2_WP3 Year 1 Report v1.0). To summarise; soil CO_2 fluxes were measured close to the static chamber location using an infra-red gas analyser (IRGA) connected to a soil respiration chamber (PP Systems, Amesbury, MA). Measurements of soil CH_4 and N_2O fluxes were made using a static chamber method (approx 30 litres) with the addition of a vent to compensate for pressure changes within the chamber during times of sampling. Chambers were enclosed for approximately 50 minutes, with four 10 ml gas samples taken over this time. Gas samples from Lincolnshire, West Sussex and Aberystwyth (all sub-sites) were analysed by gas chromatograph (GC) at the Centre for Ecology & Hydrology (CEH) Lancaster; samples from the CEH Edinburgh and Forest Research (FR) sites were analysed at their respective facilities by GC. Ancillary data consisted of measurements of volumetric soil moisture taken at three points around each chamber (ML2x Theta probe (Delta T Devices, Cambridge, UK), and of air and soil temperature (0-10 cm depth mini immersion thermometer, Testo Ltd, Alton, UK).

4.2.2. Soil chemistry

Soil %C, %N, bulk density (BD) and pH were estimated for each sub-site at each network site. These results were delivered through WP2 and the methods used are described in deliverable D2.2 (BI1001_PM04.2.2_WP2 Year 1 Chronosequence Report v1.0). Sampling

of network sites took place in Year 2 of the WP2 chronosequence sampling, with the exception of Lincolnshire, which was completed in Year 1.

4.2.3. Litter quantity

The litter layer under the crops at each network site was quantified through WP2 at the time of soil sampling using 0.25 m^2 quadrats. Seasonal litter fall was measured at a number of the network sub-sites with measurements from the SRC willow and *Miscanthus* bioenergy crops. Trays of a known dimension were placed under the crop near chamber locations and litter was collected on monthly sampling visits. This was air-dried to a constant weight, with litter input each month estimated using the dry weight and litter tray area.

4.2.4. Data and statistical analysis

Fluxes of CO_2, CH_4 and N_2O were converted into CO_2 equivalents (eq.) using GWPs of 23 and 296 for CH_4 and N_2O respectively (IPCC, 2001). Significant differences in total GHG fluxes (IRGA and chamber) were determined between the different land-uses at each site using linear mixed effects models with 'field location' (chamber number) as a random effect to account for repeated measures over time. The effect of year or season was also tested in order to determine if there were annual or seasonal effects on the differences observed between land-use. Significant differences were accepted when P values <0.05 were observed. Data was log- or square-root- transformed in order to meet assumptions of normality and homogeneity of variance. Months where data was missing from one land-use type were removed from the analyses in order to avoid un-balanced design.

In addition, to aid discussion, fluxes of IRGA CO_2 were partitioned into microbial (heterotrophic) and plant (autotrophic) respiration. Source partitioning was applied based upon a literature review completed as part of Deliverable D4.3 and on additional experiments undertaken at selected network sites. A full description of the partitioning applied within the model can be found in Deliverable D4.3. Total GHG fluxes were calculated using these heterotrophic-derived CO_2 fluxes and significant differences in fluxes between the land uses at each site were determined using linear mixed effects models with 'field location' (chamber number) as a random effect to account for repeated measures over time.

4.3. Results

4.3.1. Soil properties across the network sites

There was considerable variation in total soil C and N between the network sites, potentially reflecting soil type, management regimes and previous land use (Table 4.1). In particular the Aberystwyth site had higher soil C and N concentrations and lower bulk density than the other network sites. Soil pH was close to neutral for all network sites.

4.3.2. Litter quantity across the network sites

Table 4.1 gives an overview of the quantity of litter and coarse wood debris from each network site as determined from the litter layer sampled.

Table 4.1: Mean (± SD) %C (n=15), %N (n=15), soil bulk density (n=15) and pH values (n=5) for all network sites at 0-15 cm depth and measurements of litter-layer weight.

	Total % C	Total % N	BD	pH	Litter (t dry mass ha $^{-1}$)	
Network site / Sub site					**Leaf/Un-differentiated**	**Coarse woody**
Aberystwyth						
Miscanthus (A)	5.68 (0.62)	0.59 (0.04)	0.59 (0.12)	6.65 (0.07)	0.17 (0.08)	0
Grass(B)	6.19 (1.20)	0.63 (0.10)	0.63 (0.16)	6.44 (0.13)	0.25 (0.13)	0
East Grange						
SRF (A)	1.95 (0.52)	0.24 (0.08)	1.18 (0.11)	6.50 (0.2)	0.37 (0.27)	0.16 (0.57)
Grass (B)	2.24 (0.22)	0.23 (0.02)	1.20 (0.09)	6.74 (0.07)	1.27 (3.46)	0
SRC willow (C)	3.02 (0.43)	0.22 (0.02)	1.10 (0.10)	6.07 (0.23)	0.82 (0.31)	0.14 (0.13)
Arable (D)	2.08 (0.21)	0.24 (0.02)	1.04 (0.16)	6.83 (0.04)	0.57 (0.53)	0
West Sussex						
SRC willow (A)	1.72 (0.33)	0.19 (0.05)	1.13 (0.14)	6.04 (0.25)	1.60 (0.70)	0.04 (0.07)
Grass (B)	3.02 (0.63)	0.28 (0.04)	0.97 (0.15)	6.81 (0.23)	0.18 (0.11)	0
Lincolnshire						
Miscanthus (A)	1.81 (0.37)	0.29 (0.03)	1.38 (0.21)	7.35 (0.20)	4.51 (2.98)	3.17 (2.02)
SRC willow (B)	1.74 (0.34)	0.26 (0.03)	1.36 (0.17)	6.71 (0.13)	3.36 (2.11)	2.30 (1.49)
Arable (C)	1.89 (0.29)	0.29 (0.04)	1.13 (0.17)	6.60 (0.13)	0.82 (0.36)	0

As the longest established site, Lincolnshire bioenergy crops show the highest levels of litter and woody debris accumulation compared to the other network sites. For sites with an arable to bioenergy transition (Lincolnshire and East Grange sub C and D), the arable has less litter debris and no coarse woody debris (as expected) compared to the bioenergy crops. For the three sites with a grass to bioenergy conversion (Aberystwyth, West Sussex and East Grange A and B) the results are mixed: the grass at West Sussex had a lower amount of litter compared to the bioenergy crop whereas, at the other two sites, the grass had more litter. This is likely to be due to the difference in the age of the bioenergy crops and species differences. The *Miscanthus x giganteus* at Aberystwyth was only established in mid-2012 and litter accumulation would be expected to be low in the early stages of establishment. Species differences between the SRC willow and SRF may explain the lower

level of litter accumulation with deciduous and coniferous species exhibiting different growth strategies and patterns in litter production.

Seasonal patterns in litter fall are shown in Figure 4.1, and these reflect the different growth patterns of SRC willow and *Miscanthus x giganteus*, with litter fall occurring around four months earlier in the SRC willow. In 2011-12, estimated total litter fall at the Lincolnshire site was 371 and 191 g dwt m^{-2} for *Miscanthus x giganteus* and SRC willow respectively. Similarly for the same period at East Grange the SRC willow litter fall was 198 g dwt m^{-2}. At the Lincolnshire site in the 2012-13 measurement period total SRC willow litter fall was similar to the previous year (182 g dwt m^{-2}) whilst the total litter fall in the *Miscanthus x giganteus* was clearly lower (291 g dwt m^{-2}) when compared to the previous year; presumably due to the effect of the harrowing that year. Total litter fall from the West Sussex SRC willow was 291 and 336 g dwt m^{-2} for 2012 and 2013 respectively.

The total litter fall for two years of measurement at the Aberystwyth *Miscanthus* genotype experiment is shown in Figure 4.2. The *Miscanthus x giganteus* genotype had higher litter fall than was observed with the same genotype at the Lincolnshire field site, and in general litter fall was higher for all the genotypes when compared to the Lincolnshire crop. There was considerable variation between quantities of litter dropped from different genotypes although there was high variability between replicate plots. In the 2012-13 period lower quantities of litter were collected from *Sinensis 1* (*Sin 1*) and *Sacchariflorus 2* (*Sacc 2*) compared to the other genotypes, whereas in the 2013-14 period litter fall was more similar between the genotypes.

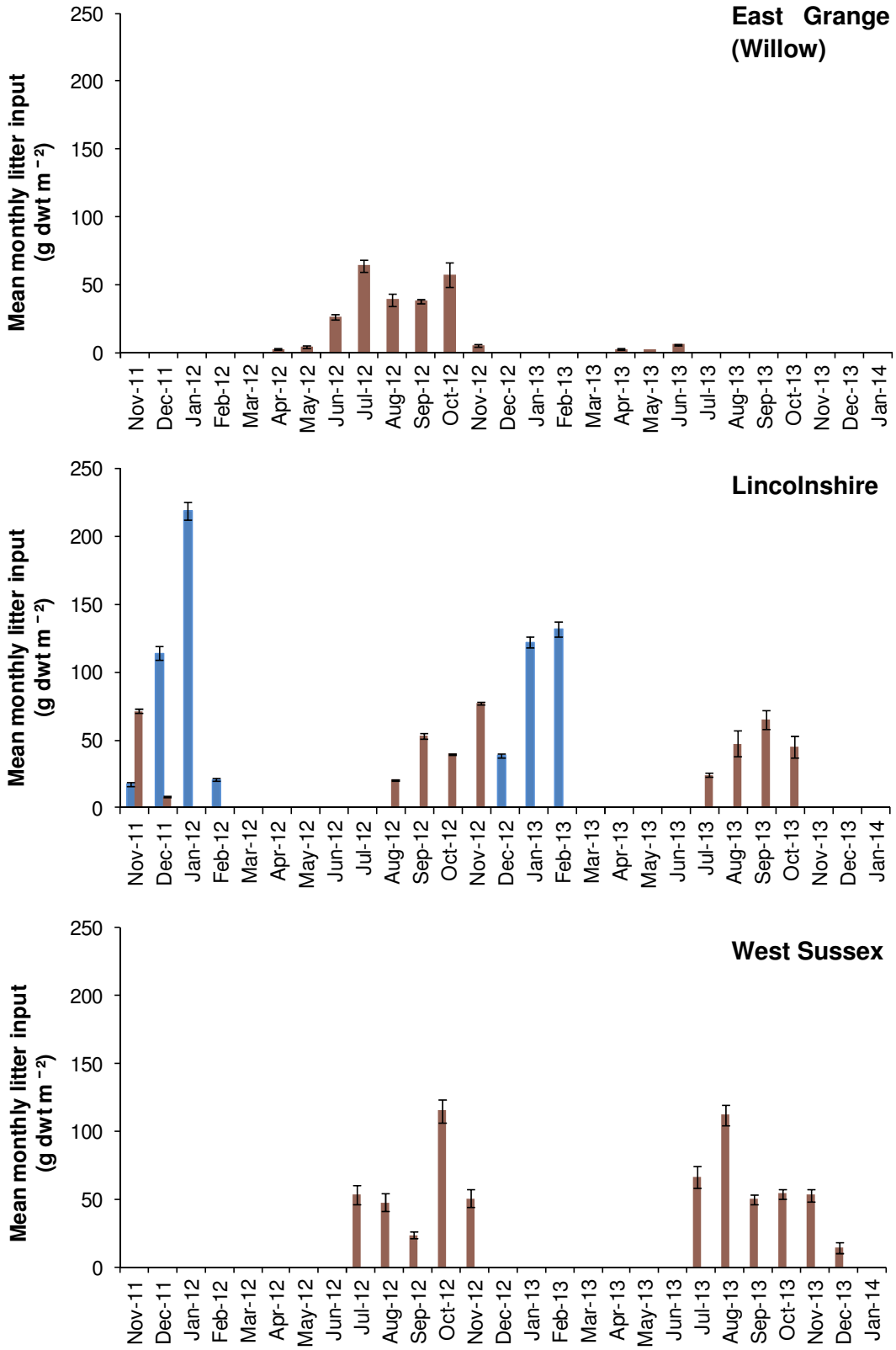

Figure 4.1: Estimated monthly patterns and quantities of litter fall for bioenergy crops at East Grange sub-site C, Lincolnshire (Sub-sites A and B) and West Sussex (Sub-site A). (Values shown are means ± 1std err (n=8))
Crop: *Miscanthus x G.* SRC willow

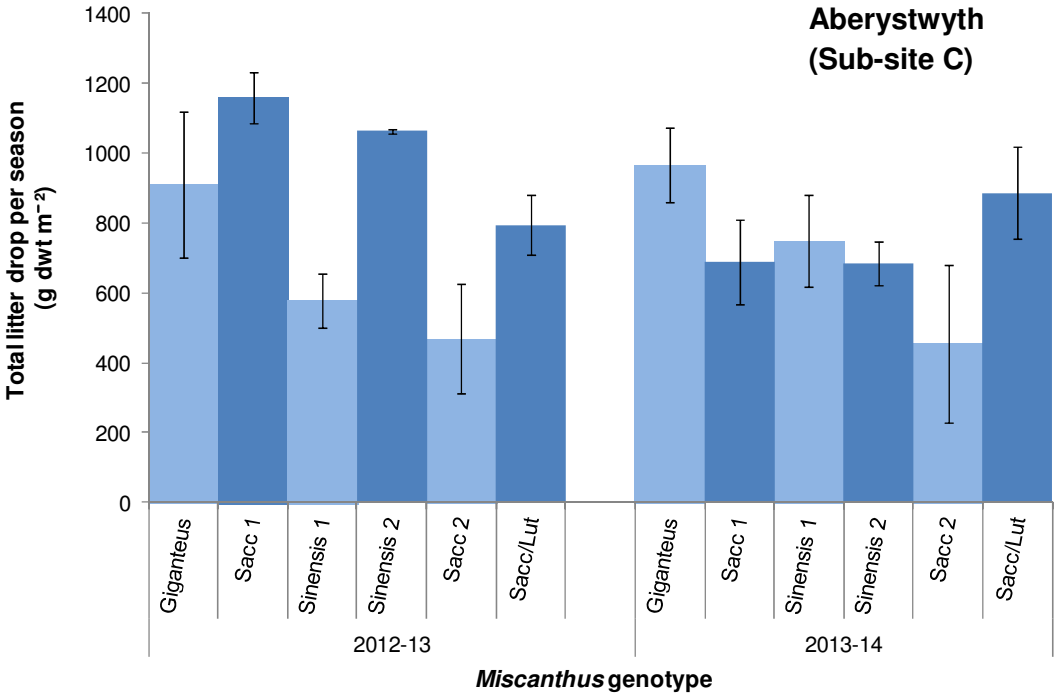

Figure 4.2: Total litter drop for the six *Miscanthus* genotypes examined at the Aberystwyth genotype experiment. Totals were estimated from monthly collections of litter over the October to January period (Values displayed are mean ± 1 std err (n=3)). Abbreviated genotype names are Sacchariflorus (Sacc 1 & 2) and the hybrid Sacc/Lutarioparis (Sacc/Lut).

4.3.3. Chamber and IRGA GHG fluxes across the network sites

The data reported in this section relate to the measurements delivered to the LUC and crop-management model (Deliverable 4.3) with no adjustments made with regard to source partitioning of soil respiration. Therefore, in the case of grass land-use the CO_2 fluxes are total ecosystem respiration (TER) because above-ground grass respiration is included whilst, in the other land-uses, fluxes are soil CO_2. The reason for this discrepancy was due to the dense sward present at the grass sites which meant that measuring soil respiration from bare patches was not possible. At the bioenergy sites, under the plant canopy the soil generally had a patchy covering of weeds and it was possible to measure bare soil respiration. For all sites CH_4 fluxes were negligible and although fluxes were used within the total GHG calculation, the CH_4 data is not shown in the total GHG flux graphics for ease of viewing.

Average GHG fluxes over both years of the measurement period are shown in Tables 4.2 and 4.3 in order to demonstrate the potential differences in fluxes between the sites and the variability in the measurements.

Table 4.2: Summary of GHG fluxes (determined from IRGA/chamber measurements only) for each crop at each network site. CH_4 and N_2O fluxes were converted into CO_2 equivalents (CO_2 eq.) using global warming potentials (GWP) of 23 and 296 for CH_4 and N_2O respectively (IPCC, 2001). Data reported are the mean fluxes (± 1 std err) from a year of monthly measurements. Yr 1 is the first twelve months of measurement at a particular site and Yr 2 is the second set of 12 months. Where negative values are reported (for example for CH_4) this signifies overall sequestration whilst positive values represent emissions of the reported gas. Data from the East Grange (Arable-Bioenergy transition) are not included as the dataset was incomplete (see page 49).

Network Site	Year	CO_2 Flux (mg CO_2 m^{-2} h^{-1})	CH_4 Flux (mg CO_2 eq. m^{-2} h^{-1})	N_2O Flux (mg CO_2 eq. m^{-2} h^{-1})	GHG TOTAL (mg CO_2 eq. m^{-2} h^{-1})
Arable vs. Bioenergy					
Lincolnshire					
Arable	Yr 1	272 ± 18	-0.15 ± 0.10	8.03 ± 2.57	**280 ± 18**
	Yr 2	252 ± 8	0.55 ± 0.49	24.1 ± 4.40	**272 ± 9.0**
Miscanthus G.	Yr 1	207 ± 14	0.04 ± 0.03	0.02 ± 0.09	**207 ± 14**
	Yr 2	295 ± 25	0.05 ± 0.08	1.70 ± 0.10	**296 ± 25**
SRC willow	Yr 1	320 ± 10	-0.03 ± 0.07	0.53 ± 0.47	**321 ± 10**
	Yr 2	304 ± 18	-0.06 ± 0.03	0.47 ± 0.17	**304 ± 18**
Grass vs. Bioenergy					
Aberystwyth					
Grass	Yr 1	974 ± 18	1.81 ± 2.23	14.4 ± 3.29	**990 ± 18**
	Yr 2	910 ± 34	-0.45 ± 0.04	2.91 ± 0.39	**913 ± 34**
Miscanthus G.	Yr 1	544 ± 45	17.5 ± 17.8	48.6 ± 11.0	**610 ± 41**
	Yr 2	493 ± 45	-0.39 ± 0.08	16.5 ± 3.24	**509 ± 44**
E. Grange FR					
Grass	Yr 1	475 ± 49	-0.03 ± 0.01	0.98 ± 0.08	**476 ± 49**
	Yr 2	443 ± 52	-0.06 ± 0.02	0.98 ± 0.14	**444 ± 52**
Short rotation forestry (SRF)	Yr 1	431 ± 59	-0.08 ± 0.01	0.83 ± 0.05	**432 ± 59**
	Yr 2	403 ± 60	-0.04 ± 0.01	1.02 ± 0.23	**404 ± 60**
West Sussex					
Grass	Yr 1	637± 49	0.08 ± 0.04	1.71 ± 0.34	**639 ± 48**
	Yr 2	629 ± 45	0.22 ± 0.18	2.62 ± 0.90	**632 ± 45**
SRC willow	Yr 1	412 ± 26	-0.10 ± 0.02	1.44 ± 0.30	**413 ± 26**
	Yr 2	337 ± 21	-0.20 ± 0.04	1.68 ± 0.29	**339 ± 21**

Table 4.3: A summary of GHG fluxes (determined from IRGA/chamber measurements only) for the genotype experiment at Aberystwyth sub-site C. Data reported are the GHG fluxes from four *Miscanthus* genotypes and a grass reference, along with soil CO_2 fluxes from a further two *Miscanthus* genotypes. CH_4 and N_2O fluxes were converted into CO_2 equivalents (CO_2 eq.) using global warming potentials (GWP) of 23 and 296 for CH_4 and N_2O respectively (IPCC, 2001). Data reported are the mean fluxes (± 1 std err) from a year of monthly measurements.

Genotype plots (Aberystwyth Sub-site C)					
		CO_2 Flux (mg CO_2 m^{-2} h^{-1})	CH_4 Flux (mg CO_2 eq. m^{-2} h^{-1})	N_2O Flux (mg CO_2 eq. m^{-2} h^{-1})	**GHG TOTAL** (mg CO_2 eq. m^{-2} h^{-1})
Grass	Yr 1	348 ± 23	-0.37 ± 0.07	2.44 ± 0.53	**351 ± 23**
	Yr 2	435 ± 34	-0.18 ± 0.03	1.26 ± 0.5	**436 ± 34**
Giganteus	Yr 1	344 ± 11	-0.11 ± 0.17	2.76 ± 0.49	**347 ± 11**
	Yr 2	283 ± 24	-0.39 ± 0.05	1.99 ± 0.59	**279 ± 29**
Sacc 1	Yr 1	373 ± 33	-0.26 ± 0.02	2.51 ± 0.28	**375 ± 33**
	Yr 2	418 ± 18	-0.18 ± 0.02	1.17 ± 0.47	**419 ± 19**
Sinensis 1	Yr 1	322 ± 34	-0.12 ± 0.14	3.86 ± 1.83	**326 ± 32**
	Yr 2	281 ± 26	-0.17 ± 0.05	2.12 ± 1.66	**283 ± 27**
Sinensis 2	Yr 1	272 ± 41	-0.33 ± 0.05	2.40 ± 0.78	**274 ± 41**
	Yr 2	320 ± 30	-0.33 ± 0.11	1.14 ± 0.48	**326 ± 35**
Sacc/Lut	Yr 1	553 ± 33	No Data	No Data	
	Yr 2	263 ± 31	No Data	No Data	
Sacc 2	Yr 1	348 ± 29	No Data	No Data	
	Yr 2	304 ± 34.2	No Data	No Data	

4.3.3.1. Aberystwyth (Penglais sub-sites A and B)

The design of the experiment of the Penglais site allowed the effects of planting of *Miscanthus x giganteus* on GHG dynamics to be monitored throughout the land-use transition period (Figure 4.3). In order to perform statistical comparisons of the effects of planting, the dataset was split into pre- and post- establishment. A further subset of data was taken from June-December of each year in order to ensure balanced design when comparing measurements from Year 1 against Year 2. Prior to the planting of *Miscanthus x giganteus* there was no significant difference in overall GHG emissions between the grass sites although N_2O emissions were significantly higher from the pre- *Miscanthus x giganteus* plots.

Following conversion to *Miscanthus x giganteus*, total soil GHG fluxes (June 2012 to December 2013) were found to be significantly lower in the *Miscanthus x giganteus* fields when compared to those fields left as grass ($F_{(1,14)}$ 62.1, $P<0.001$). This was observed for both the growing season following transition from grass to *Miscanthus x giganteus* and in the second year after transition, with no significant difference in emissions between the two years of measurements.
CO_2 was the primary contributor to GHG emissions from both the grass and *Miscanthus x giganteus* sites although significant N_2O emissions were observed from both land-uses on a number of sampling occasions. N_2O production was significantly higher from the fields used for *Miscanthus x giganteus* ($F_{(1,14)}$ 54.7, $P<0.001$), however N_2O fluxes were originally higher from those plots when both fields were grass. CH_4 fluxes were generally around zero (Table 4.2) with high variation within the land-use replicates. The high mean value recorded for *Miscanthus x giganteus* in 2012 is driven by one replicate with very high CH_4 production and there was large standard error associated with the measurements within land-use type. In general CH_4 production or consumption was negligible for both land-uses, with no crop specific controls on CH_4 fluxes evident.

4.3.3.2. Aberystwyth Sub-site C (Genotype plots)

CO_2 and N_2O fluxes for the first year of measurement (Nov 11-Oct 12) are shown in Figure 4.4a with the second year of measurement (Nov 12-Oct 13) shown in Figure 4.4b. There was no significant difference in GHG fluxes measured from the grass reference and the four *Miscanthus* genotypes, nor were there significant differences between the different genotypes. CO_2 production was the predominant GHG flux measured with N_2O production occasionally observed in low quantities and with no clear pattern with regard to crop type. A further two *Miscanthus* genotypes were sampled using an IRGA for soil CO_2 flux from June 2012-Oct 2013. No significant difference in CO_2 flux was found between the different genotypes, nor with the grass reference. A statistical analysis of the relationship between the N_2O emissions and the soil moisture measurements found no significant relationship.

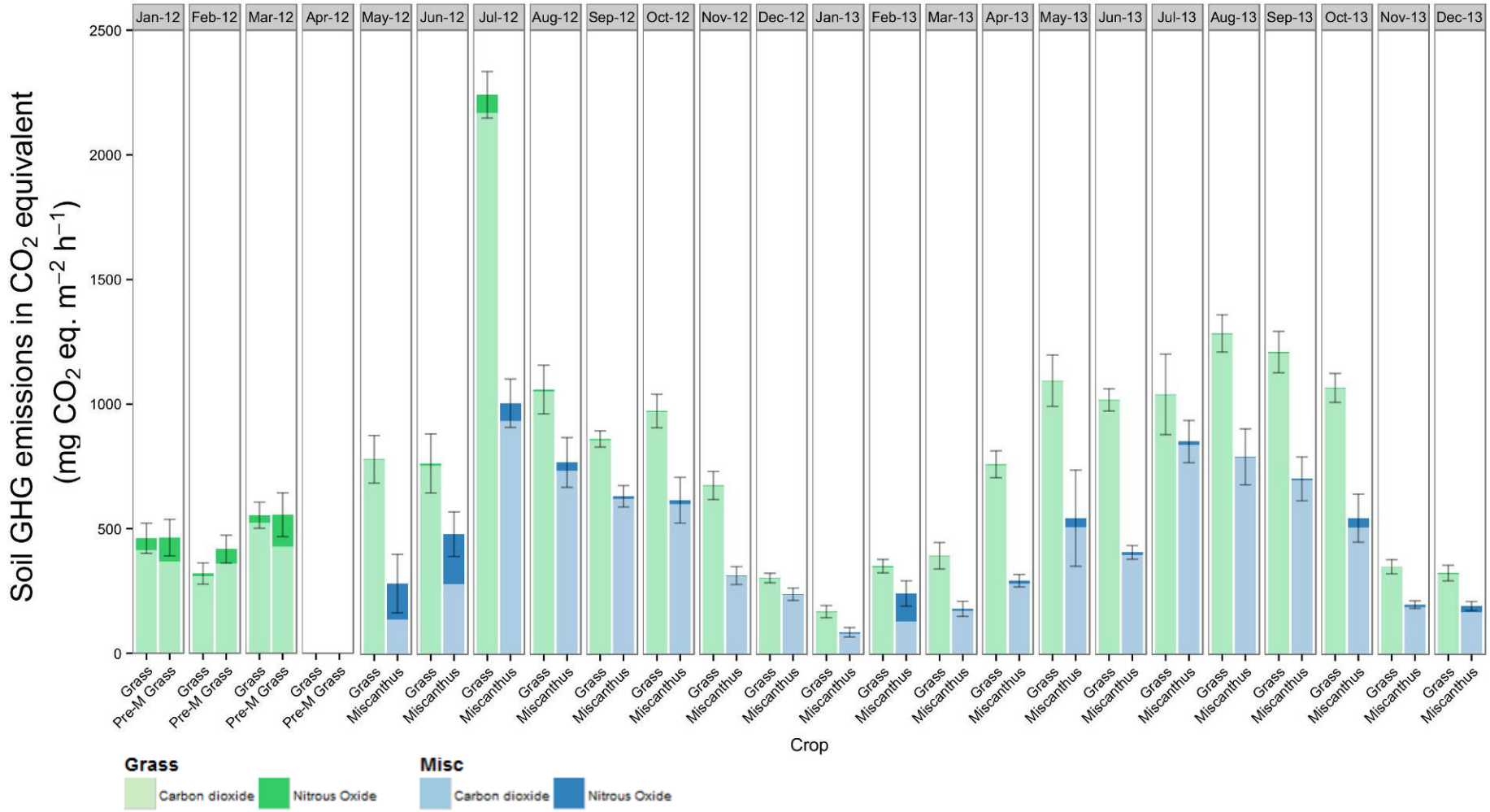

Figure 4.3: CO_2 and N_2O fluxes (in CO_2 eq) from the land-uses examined at Penglais, Aberystwyth from Jan 2012-Dec 2013 measured monthly. Grass fluxes represent TER whilst for the *Miscanthus x giganteus* soil fluxes are reported. Values reported are means (± 1 std err (n=8)). GHG emissions were significantly higher from the grass plots compared to the *Miscanthus x giganteus* plots (*P < 0.001)*

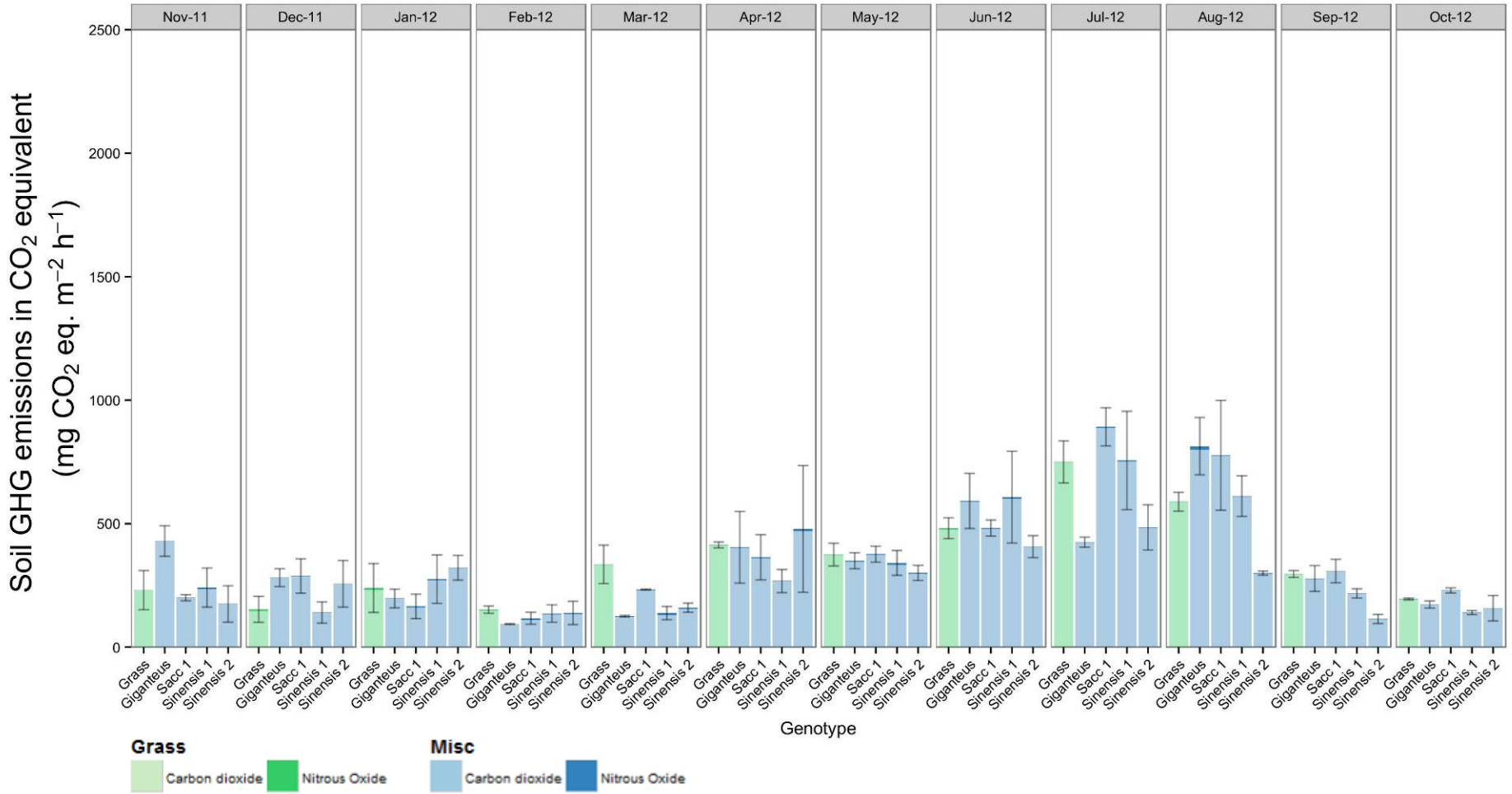

Figure 4.4a: CO_2 and N_2O fluxes (in CO_2 eq) from the grass reference and four genotypes of *Miscanthus* (Aberystwyth Sub-site C) from Nov 2011-Oct 2012 measured monthly. Grass fluxes represent TER whilst for the *Miscanthus* soil fluxes are reported. Values reported are means (± 1 std err, (n=8)). There were no significant differences in measured GHG emissions between the Miscanthus genotypes, nor between the *Miscanthus* genotypes and the reference land use, grass.

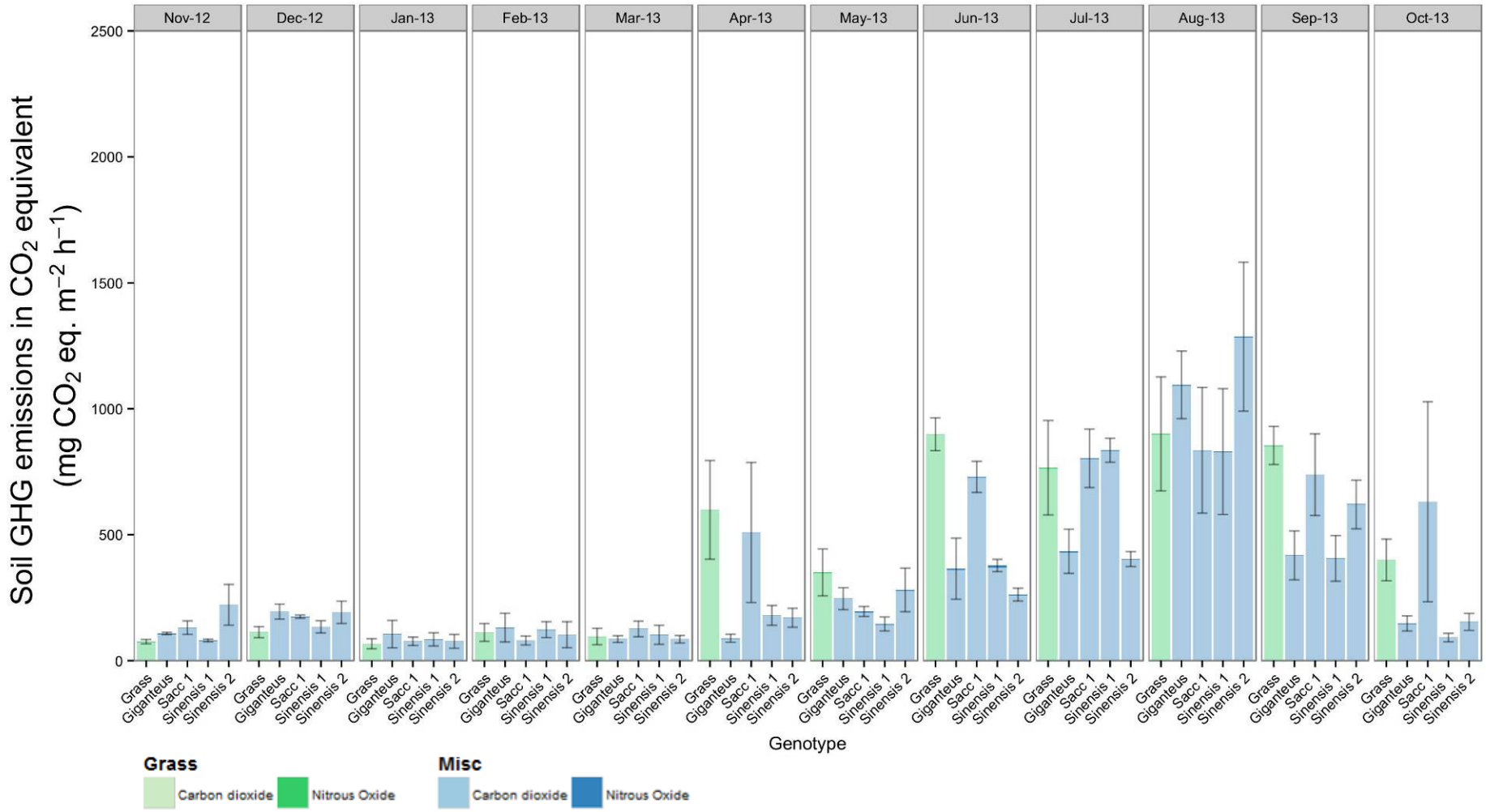

Figure 4.4b: CO_2 and N_2O fluxes (in CO_2 eq) from the grass reference and four genotypes of *Miscanthus* (Aberystwyth Sub-site C) from Nov 2012-Oct 2013 measured monthly. Grass fluxes represent TER whilst for the *Miscanthus* soil fluxes are reported. Values reported are means (± 1 std err, (n=3)). There were no significant differences in measured GHG emissions between the Miscanthus genotypes, nor between the *Miscanthus* genotypes and the reference land use, grass.

4.3.3.3 .East Grange, Fife

The ELUM site at East Grange consists of four sub-sites; with sampling of the grass and SRF by Forest Research, and sampling of the arable and SRC willow sub-sites by CEH Edinburgh. Due to the different sampling times, and (in the case of the arable-SRC willow dataset) a number of months when sampling could not take place, it was not possible to make a direct comparison between the data sets from Forest Research and CEH Edinburgh.

Grass to SRF conversion (East Grange)

Across the sampling period (Jan 2012-Dec 2013) there was no significant difference between GHG fluxes observed from the grass and SRF sub-sites (Figure 4.5). CO_2 emissions were the primary contributor to the GHG flux measured with negligible fluxes of CH_4 and small amounts of N_2O production (Table 4.2).

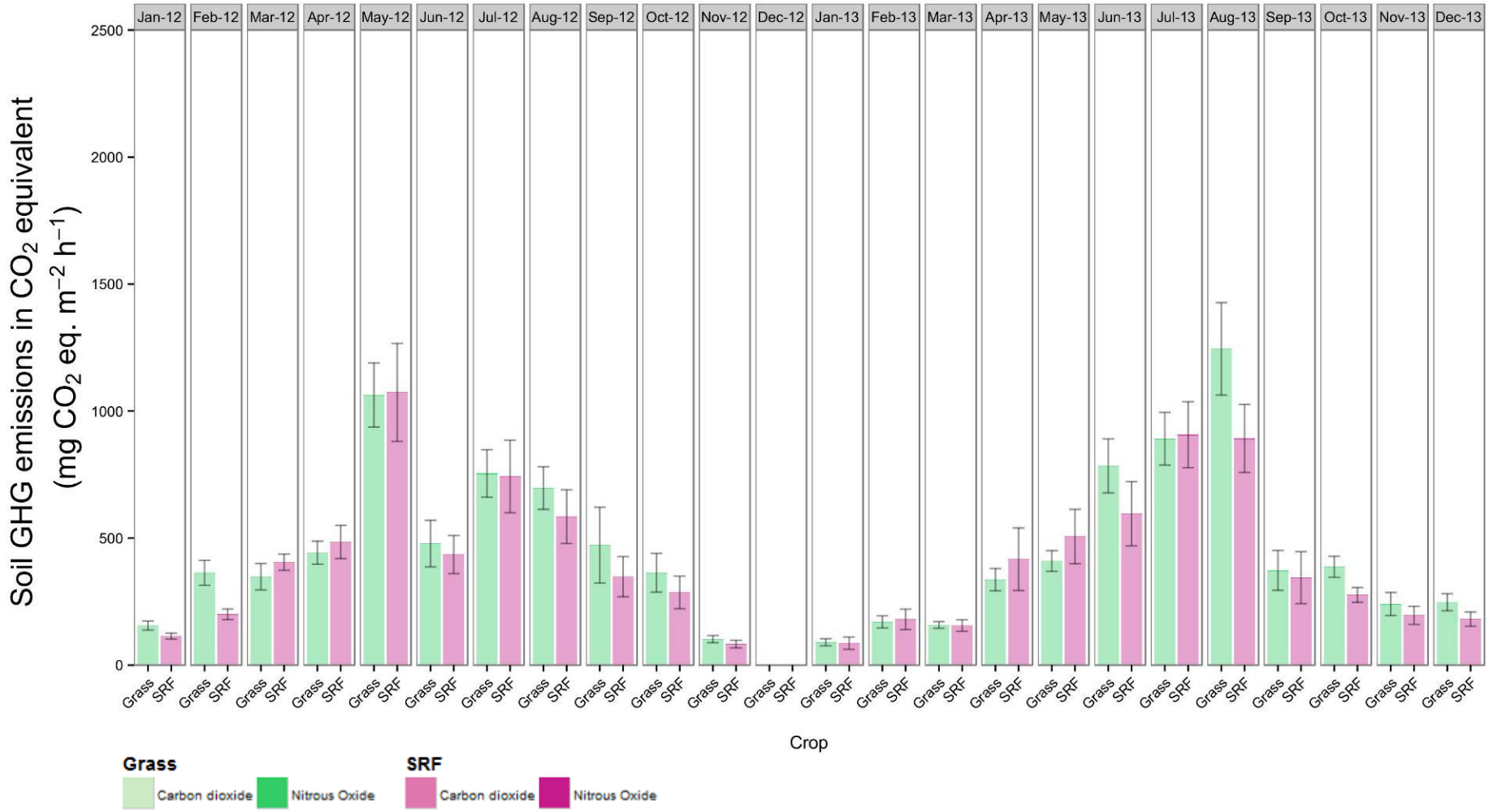

Figure 4.5: CO_2 and N_2O fluxes (in CO_2 eq) from the grass and SRF land-uses examined at East Grange, Fife from Jan 2012-Dec 2013 measured monthly. Grass fluxes represent TER whilst SRF fluxes represent soil and grass respiration. Values reported are means (± 1 std err, (n=8)). There was no significant difference in measured GHG emissions between the SRF and the reference land use, grass.

Arable to SRC willow conversion (East Grange)

Due to technical issues and issues regarding access to sites for sampling, the dataset for the arable to SRC willow conversion at East Grange is missing a substantial number of months (Figure 4.7). The approach to analysing the dataset was to only use data from the months for which GHG flux data was available for both crops; however, caution should be applied when evaluating the results as significant sections of the growing season for both the arable and SRC willow crops are missing. Over the months where a direct comparison could be made between land-use there was no significant difference observed in total GHG fluxes.

CO_2 was the primary GHG emitted from both the SRC willow and the arable soils with N_2O production found to be significantly higher in the arable field ($F_{(1,18)}$ 150, $p< 0.001$). Trends in N_2O production were not found to be linked to environmental factors and are most likely driven by fertiliser addition (Figure 4.6). The emissions of N_2O from the SRC willow soils were negligible with fluxes around zero on the majority of sampling occasions.

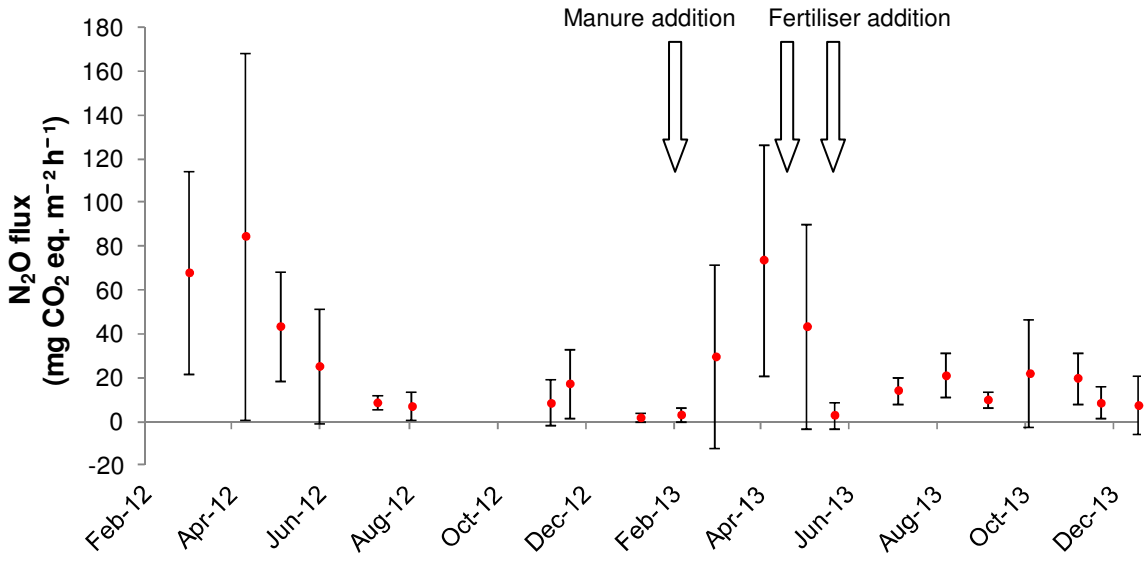

Figure 4.6: Soil N_2O fluxes (in CO_2 eq) from the arable land-use at the East Grange site from March 2012-January 2014 measured monthly. Soil N_2O fluxes from the arable are represented by the red circles. Values reported are means (± 1 std err, (n=8)).

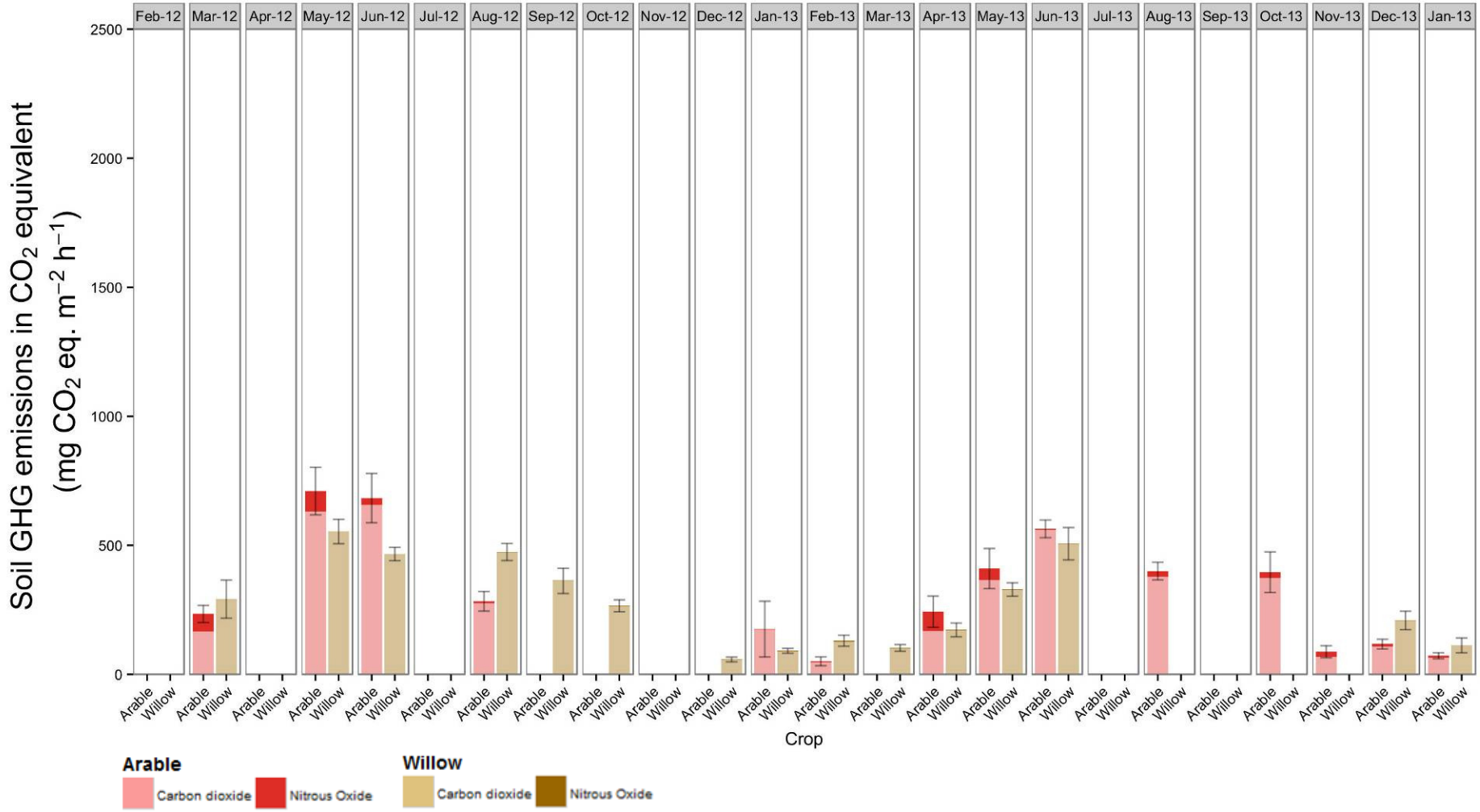

Figure 4.7: Soil CO_2 and N_2O fluxes (in CO_2 eq) from two of the land-uses examined at East Grange, Fife from Feb 2012-Jan 2014 measured monthly. Values reported are means (± 1 std err, (n=10))

4.3.3.4. Lincolnshire

Across the period Jan 2012-Dec 2013, significantly lower soil GHG emissions were measured from the *Miscanthus x giganteus* compared to the SRC willow and the arable *(*$F_{(2,21)}$ 5.17, *P <0.05*). Soil GHG fluxes were lower in the *Miscanthus x giganteus* compared to the arable in 2012, but not in 2013. This could be linked to the management regime applied to the *Miscanthus x giganteus* in 2013 which included: harrowing to break up the rhizomes and the application of wood-waste. These management practices might be expected to affect microbial activity and increase soil respiration rates. EC data, reported in Section 3 of this report, showed that respired CO_2 over the *Miscanthus x giganteus* was higher in 2013 compared to 2012. Chamber measurements did show that *Miscanthus x giganteus* CO_2 fluxes were lower in 2012 than 2013, but the variability within the field measurements was also high and the difference was not significant.

Throughout the sampling period, CO_2 was the primary contributor to soil GHG emissions across the three land-uses examined (Figure 4.9). In the arable, fluxes of N_2O on average contributed 3 and 9% to the soil GHG emissions over 2012 and 2013 respectively. Figure 4.8 shows that the fluxes of N_2O in the arable were irregular but, as shown in Figure 4.9, N_2O emission could contribute over 50% of the soil GHG emission at times when soil respiration was low (for example Nov and Dec 13). It is likely that N_2O emissions were driven by management practices in the arable, such as fertiliser application, with N fertiliser applications in April/May 2012 and April-June 2013. Soil CH_4 fluxes were negligible at the Lincolnshire site for the three land-uses examined (Table 4.2).

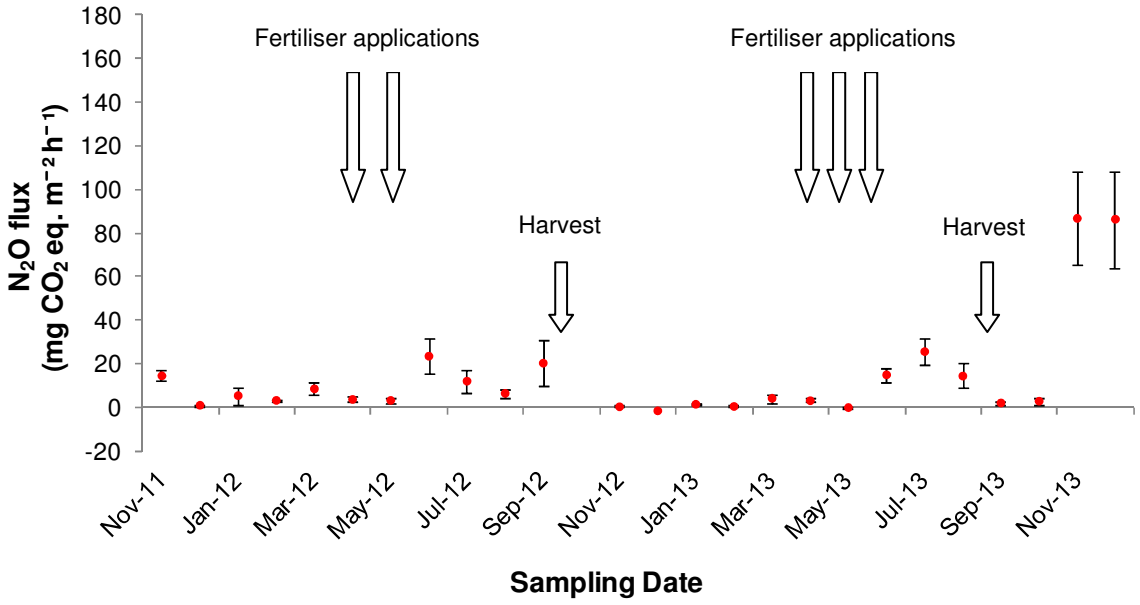

Figure 4.8: Soil N_2O fluxes (in CO_2 eq) from the arable land-use at the Lincolnshire field site from Nov 2011-Dec 2013 measured monthly. Soil N_2O fluxes from the arable are represented by the red circles. Values reported are means (± 1 std err, (n=8)).

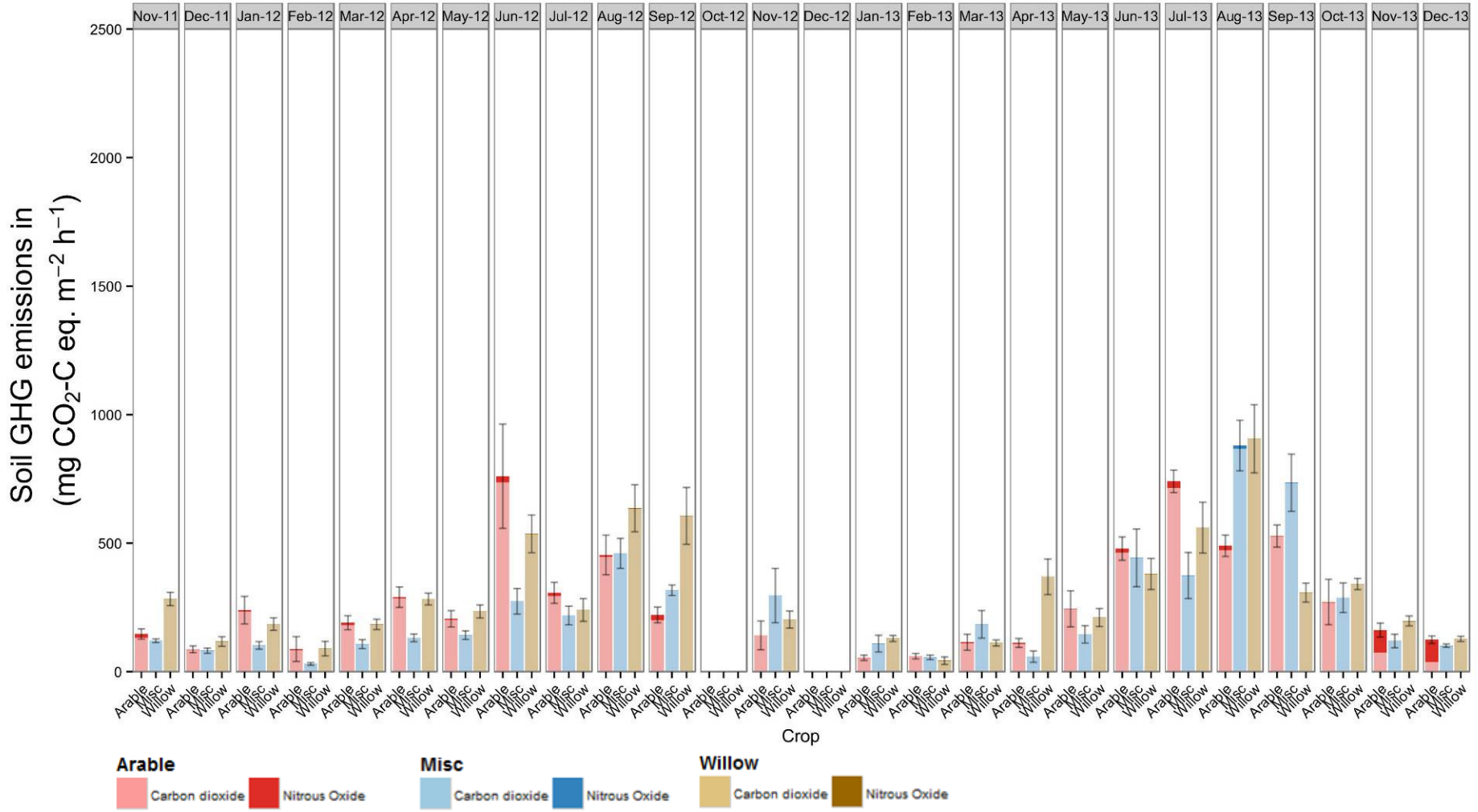

Figure 4.9: Soil CO_2 and N_2O fluxes (in CO_2 eq) from the three land-uses examined at the Lincolnshire field site from Nov 2011-Dec 2013 measured monthly. Values reported are means (± 1 std err, (n=8)). Soil GHG emissions were significantly lower in the *Miscanthus x giganteus* compared to the arable and SRC willow ($F_{(2,21)}$ 5.17, $P < 0.05$)

4.3.3.5. West Sussex

CO_2 was the dominant GHG emission observed across both land-uses during the sampling period, whilst fluxes of CH_4 and N_2O were generally low (Figure 4.11). There was significantly higher ecosystem CO_2 emission from the grass land-use when compared to the SRC willow soil respiration ($F_{(1,22)}$ 37.91, *P<0.001*) and this was observed across both measurement years. GHG emissions were slightly lower from both crops in the second year of measurement.

N_2O fluxes were low in comparison to CO_2 emissions, however occasional fluxes were observed from both land-uses with no important differences between the sites. CH_4 fluxes tended to be close to zero although generally negative in the SRC willow (indicating net CH_4 oxidation) and positive in the grass (indicating net CH_4 production). Emissions of CH_4 were observed from the grass land-use at a number of sampling occasions however variability was high between the replicate chambers in the crop and it was not possible to fit a statistical model which conformed to requirements of normality and homogeneity of variance (Figure 4.10). Environmental factors, such as soil/air temperature and soil moisture were not found to correlate with the CH_4 emissions from the grass.

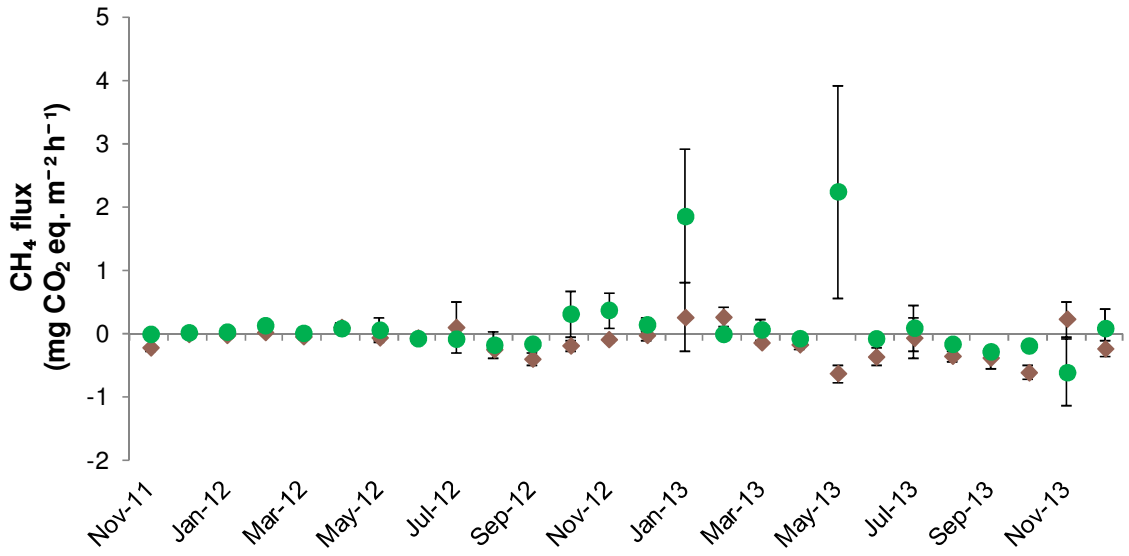

Figure 4.10: Soil CH_4 fluxes (in CO_2 eq) from the two land-uses examined at the West Sussex field site from Nov 2011-Dec 2013 measured on monthly sampling. Grass fluxes are shown by green circles whilst SRC willow is represented by brown diamonds. Values reported are means (± 1 std err, (n=8))

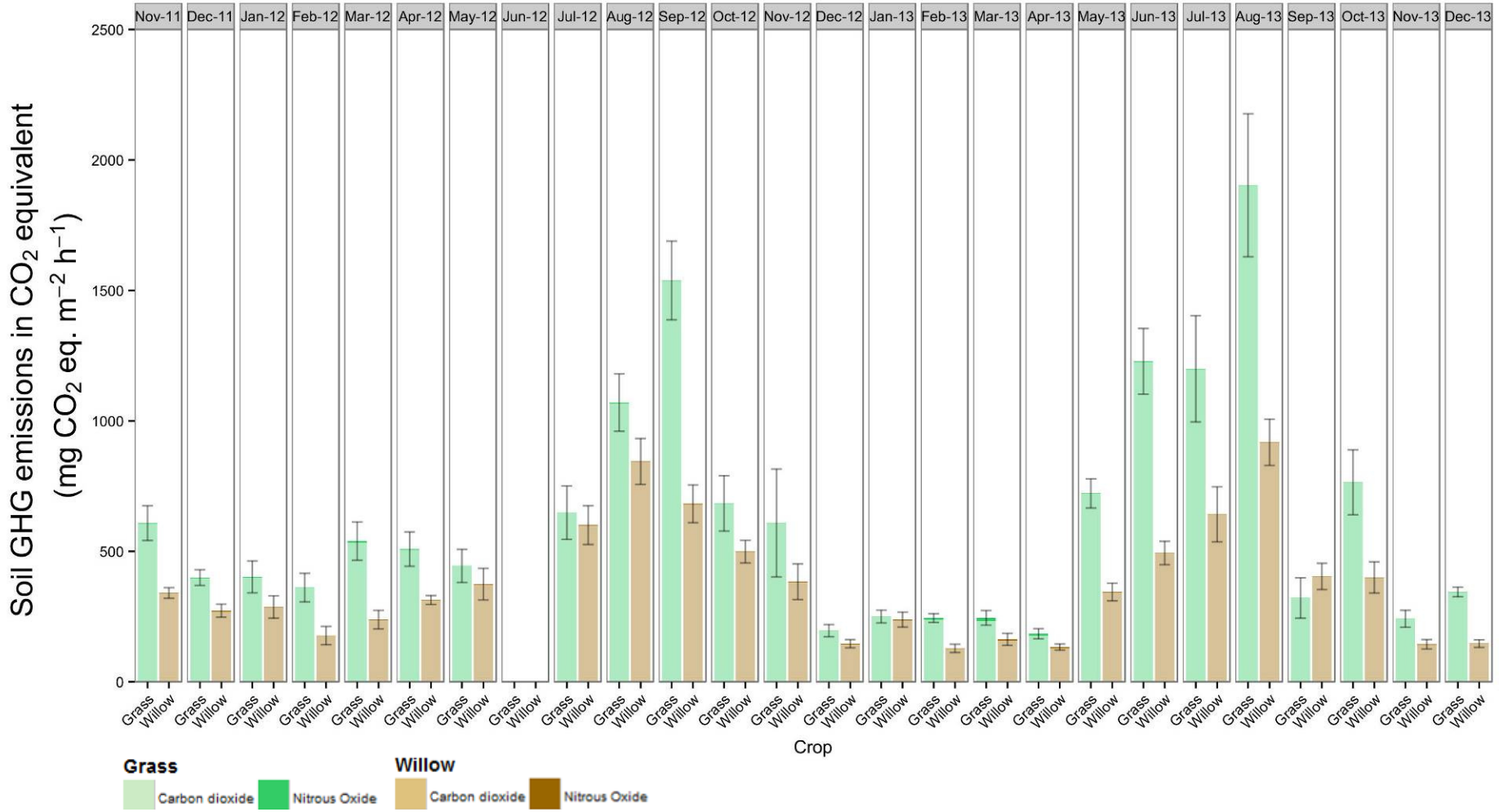

Figure 4.11: CO_2 and N_2O fluxes (in CO_2 eq) from the two land-uses examined at the West Sussex field site from Nov 2011-Dec 2013 measured on monthly sampling. Grass fluxes represent TER whilst in the SRC willow soil fluxes are reported. Values reported are means (± 1 std err, (n=8)). Measured GHG emissions were significantly higher from the grass land use compared to the willow ($F_{(1,22)}$ 37.91, *P<0.001)*

4.3.4. Source-partitioned soil respiration

In order for comparisons to be performed between the grass references and the bioenergy crops, partitioning of respiration between autotrophic and heterotrophic respiration is required. In the following section we have applied source partitioning to the soil CO_2 fluxes measured with the IRGAs. The results following partitioning are summarised below.

4.3.4.1. Aberystwyth (Sub-site A and B)

Partitioning soil CO_2 fluxes into autotrophic and heterotrophic respiration for both grass and *Miscanthus x giganteus* resulted in notably lower emission rates when specifically considering soil surface fluxes. Mean GHG fluxes of 265.1 and 216.1 mg CO_2 eq. m^{-2} h^{-1} for grass and *Miscanthus x giganteus* respectively were found for the post-planting phase. Significantly lower total GHG fluxes ($F_{(1,14)}$ 10.5, *p<0.01*) were observed from the *Miscanthus x giganteus* land-use when compared to the grass references.

4.3.4.2. Aberystwyth (Sub-site C-Genotype plots)

In this experiment, grass was trimmed within the sampling chambers in order to remove grass leaf respiration. This is reflected in the lower TER observed for the grass plots at this sub-site and in the similar CO_2 fluxes obtained through IRGA measurements on the grass vs. *Miscanthus* plots. There is still likely to be a contribution of grass root respiration to overall CO_2 flux. Therefore, source partitioning was used to separate root-derived respiration in both the *Miscanthus* and grass plots, with no adjustment made to account for above-ground grass respiration. This resulted in significantly lower soil GHG fluxes from three of the *Miscanthus* genotypes (*Giganteus, Sin 1* and *2* with fluxes of 131.3, 124.7 and 124.7 mg CO_2 eq. m^{-2} h^{-1} respectively) when compared to the grass reference ($F_{(4,10)}$ 5.52, *p<0.05*). The grass reference and the *Sacc1* genotypes had fluxes of 200.1 and 157.1 mg CO_2 eq. m^{-2} h^{-1} respectively with no significant difference in fluxes over the two years.

4.3.4.3. East Grange

Subsite A and B

The site was recently established (February 2009) and grass dominated the above-ground vegetation of the site. Therefore, respiration measured in the SRF plots includes grass leaf respiration similar to the grass reference. The CO_2 fluxes from the SRF were partitioned in a similar manner to those from the grass reference plots. Partitioning resulted in mean GHG fluxes of 140.8 and 128.8 mg CO_2 eq. m^{-2} h^{-1} from the grass and SRF plots respectively, with no significant difference in soil emissions between land-uses.

Subsite C and D

Following source partitioning of CO_2, mean soil GHG fluxes over the two years were estimated to be 79.4 and 19.5 mg CO_2 eq. m^{-2} h^{-1} for arable and SRC willow land-uses respectively. GHG fluxes from the arable soil were found to be significantly higher ($F_{(1,18)}$ 84.7, *p<0.001*) however comparison between land-use was made over a limited number of time-points due to issues with data collection, e.g. Jul.-Nov. 2013 when equipment had to be removed from the SRC willow in anticipation of the harvest which was then delayed. The result of this is that the differences observed may only be representative of the time of year

data is available, with limited data for the mid-summer to late autumn time frame. This may explain why the mean fluxes for these sub-sites are low compared to other sub-sites. At other sub-sites there were a greater number of observations from the summer months when soil GHG fluxes are generally higher, driven by warmer temperatures and higher plant photosynthesis.

4.3.4.4. Lincolnshire

Partitioning of respired CO_2 resulted in significantly lower mean GHG fluxes ($F_{(2,29)}$ 24.7, *p<0.001*) for the SRC willow (83.2 mg CO_2 eq. m^{-2} h^{-1}) and *Miscanthus x giganteus* (142.6 mg CO_2 eq. m^{-2} h^{-1}) compared to the arable sub-site (197.5 mg CO_2 eq. m^{-2} h^{-1}). This trend was observed in both years of the study.

4.3.4.5. West Sussex

Source partitioning of the CO_2 fluxes for the West Sussex site had a large impact on the overall differences between the land-uses. Source partitioning of the SRC willow was based upon root-exclusion experiments which showed that heterotrophic respiration under SRC willow contributed to around 80% of the total soil CO_2 flux. This resulted in a large proportion of the measured soil CO_2 flux being attributed to heterotrophic respiration. Mean soil GHG flux (using heterotrophic respiration) was determined as 303.0 mg CO_2 eq. m^{-2} h^{-1} for the SRC willow, whilst mean flux from the grass references was far lower (198.2 mg CO_2 eq. m^{-2} h^{-1}). Overall, using GHG fluxes determined with heterotrophic respiration, soil GHG emissions were significantly higher from SRC willow compared to the grass references ($F_{(1,22)}$ 38.7, *p<0.001).*

4.4. Discussion

4.4.1. Overview

The aim of this work was to monitor GHG emissions over different bioenergy transitions at a monthly timescale, in order to capture potential effects of land-use change on soil microbial activity. The primary purpose of this work was to deliver GHG flux data to parameterise and validate the LUC and crop management model (Deliverable D4.3). In this discussion we summarise the observed differences in GHG potentials across the land-use transitions particularly with regard to non-CO_2 GHGs. However, the conclusions drawn from this section are based on the monthly measurements and therefore provide only a snapshot of the ecosystem measured at those specific times. Therefore, conclusions for policy development should be drawn from the LUC and crop management model (see deliverable D4.6), which provides the comprehensive evaluation of GHG fluxes with regard to temporal and spatial variation.

GHG flux data is summarised in Figures 4.12 and 4.13. Dashed lines on each figure represent the 1:1 relationship, with points falling below the line representing lower fluxes in the bioenergy land-use, compared to the reference land-use, whilst points above the line represent higher fluxes. The genotype experiment at Aberystwyth is represented by the *Miscanthus x giganteus* genotype only, to allow direct comparisons between the same

genotype at different field sites. Similar patterns were observed with the other genotypes examined when compared to the grass reference.

4.4.2. Non-CO_2 GHGs

Whilst EC systems provide valuable information regarding the net flux of CO_2 from a land-use, there are few EC systems available which are able to monitor fluxes in CH_4 and N_2O. The use of chamber measurements allowed fluxes of non-CO_2 GHGs to be monitored and to determine whether annual fluxes could be modelled from the monthly data collection. The challenge of modelling CH_4 and N_2O is that emissions of these gases are highly variable in space and time, with the production and consumption processes being controlled by a large number of environmental and biological variables (Li, 2000). Monthly measurements provided an overview of the potential for the different land-uses to produce (or consume) CH_4 and N_2O, however no relationships between environmental drivers and the observed pulses of either CH_4 or N_2O were found.

The CH_4 fluxes determined were generally negligible compared to CO_2 and N_2O emissions, with high relative standard deviation within each land-use. With the exception of Penglais and West Sussex, there were no differences observed in CH_4 flux between the land-uses (Figure 4.12a). At the aforementioned sites, occasional pulses of CH_4 were observed from certain plots in the *Miscanthus x giganteus* fields at Penglais and in the grass reference at West Sussex. These pulses of CH_4 did not appear to be significantly related to land-use, nor to measured environmental parameters (soil moisture and temperature), with high within-land-use replicate variability observed. The average CH_4 emission from the Penglais *Miscanthus x giganteus* site is not reported in Figure 4.12a. The value is high compared to all other land-uses, which results in a skewed graph. Average CH_4 fluxes for that site were 0.40 and 7.32 mg-CO_2 eq. m^{-2} hr^{-1} for the grass and *Miscanthus x giganteus,* respectively. However, due to the high variability within the *Miscanthus x giganteus* plots, there was no significant difference between the bioenergy crop and the grass reference.

CH_4 oxidation is generally associated with dry, aerated soils with reduced oxidation rates observed for croplands (Dobbie *et al.*, 1996) where factors such as N fertilisation (Mosier *et al.*, 1991; Hu *et al.*, 2002) and compaction (Ball, 2013) result in conditions less favourable for CH_4 consumption (Hansen *et al.*, 1993). However, across the network sites there were no significant effects of land-use change on CH_4 oxidation observed, reflecting the current opinion that within bioenergy LCAs, CH_4 fluxes contribute relatively little to total GHG cycles (Berndes *et al.*, 2011).

N_2O fluxes were generally low from all bioenergy crops with the exception of Aberystwyth sub-sites A and B (Penglais), where there were significantly higher emissions from the *Miscanthus x giganteus* field compared to the grass reference (Figure 4.12b). It is important to note that at this site, N_2O emissions from the *Miscanthus* plots were significantly higher prior to conversion compared to the plots that remained as grass. Therefore it is not possible to attribute the higher fluxes observed post-planting solely to the transition to bioenergy. Transition of grassland to bioenergy crop has been linked to short-term increases in N_2O emissions (Nikièma *et al.*, 2012; Palmer *et al.*, 2013), with herbicide use and soil preparation found to increase available N which can be utilised by de-nitrifying bacteria (Nikièma *et al.*, 2012).

The greatest benefits regarding reduction in N_2O emissions were observed with the transition from arable land-use to bioenergy (Figure 4.12b). Perennial crops have greater N-use efficiency (Kavdir *et al.*, 2008), with *Miscanthus* and woody coppice crops shown to have more favourable impacts with regard to soil N_2O emissions when compared to crops with higher N-demand, such as oil seed rape, maize and switch-grass (Crutzen *et al.*, 2007; Davis *et al.*, 2010). N_2O emissions from arable fields have been shown to peak following fertiliser additions (Mosier *et al.*, 1991; Hansen *et al.*, 1993; Kavdir *et al.*, 2008), after-plant harvest and during freeze-thaw cycles (Kavdir *et al.*, 2008). In order to establish the drivers of N_2O emissions, Kavdir et al (2008) carried out intensive gas sampling with measurements taken four times a week. The monthly measurements performed during the ELUM sampling period provide evidence of significant reductions in N_2O emissions following the switch from arable to perennial bioenergy crop. However, measurements at greater temporal resolution are required to determine the mechanisms driving the arable N_2O fluxes and the duration of the response to fertiliser addition.

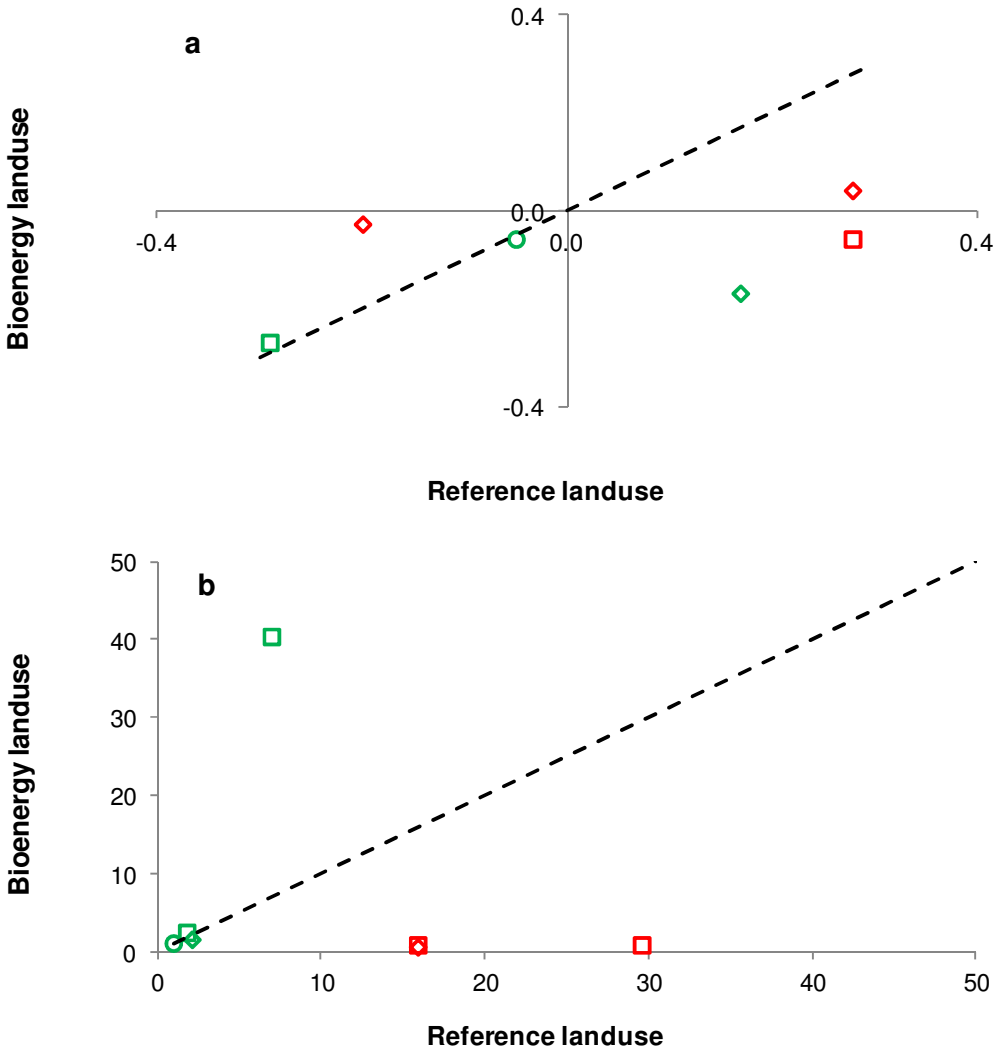

Figure 4.12: Soil fluxes of a) CH_4 (in mg-CO_2 eq. m^{-2} hr^{-1}) and b) N_2O (in mg-CO_2 eq. m^{-2} hr^{-1}) over the two-year sampling period for the reference land-use (shown as green for grass and red for arable) and the bioenergy land-use (denoted by squares for *Miscanthus x giganteus,* circles for SRF, and diamonds for SRC willow). The 1:1 relationship is shown by the dashed line.

Fertiliser applications were not made to any of the bioenergy crops presented in this report which is likely to contribute to the low N_2O emissions reported. Over the full lifetime of these energy crops it is important to acknowledge the potential for higher N_2O emissions in the later stages of the crop lifecycle. Research suggests that limited use of N fertiliser on bioenergy crops within 2-3 years of establishment would be required in order to maintain bioenergy crop productivity and ensure replacement of N removed from the system during harvest (Miguez *et al.*, 2008; Cadoux *et al.*, 2012; Finnan *et al.*, 2014). The LUC and crop management model (deliverable D4.3) accounts for this by incorporating recommended applications of N fertiliser as specified by the Defra Fertiliser Manual (RB209).

Work based on *Miscanthus* demonstrates that the balance between yield benefits and environmental impacts, in terms of N_2O emissions, is variable (Karp & Shield, 2008; Miguez *et al.*, 2008; Larsen *et al.*, 2014). This balance is controlled primarily by yield gains arising

from fertilisation, although factors determining N_2O emissions, such as environmental conditions, fertiliser application rates and N-use efficiency of different crops, will also be important. Yield gains for *Miscanthus* following N fertilisation are generally low (Miguez *et al.*, 2008; Cadoux *et al.*, 2012; Roth *et al.*, 2014) with some studies finding no significant increase in yield, even over longer timescales (14 to 20 years) (Christian *et al.*, 2008; Larsen *et al.*, 2014). The yield gains associated with SRC willow have been studied less extensively with recent work demonstrating that N fertilisation increased biomass yields by up to 35% (Finnan *et al.*, 2014). For both *Miscanthus* and SRC willow it has been observed that there is little difference in yields when comparing low and high levels of fertiliser addition, suggesting that fertiliser application should be limited to amounts sufficient to replace N lost through harvest (Cadoux *et al.*, 2012). In order to better incorporate the N_2O emission potential over the life-time of bioenergy crops it is essential for long term studies to be implemented with different fertiliser treatments applied. In addition, the determination of emission factors associated with bioenergy crops will be valuable for assessing the yield vs. N_2O benefits of different crop types and species.

4.4.3. Non-partitioned vs. partitioned respiration

The conclusions drawn from the chamber and IRGA GHG measurements are dependent on methods used to partition the respiration source. This has particularly important implications with regard to the grass references, where above-ground grass respiration contributes considerably to CO_2 fluxes measured. Figure 4.13 demonstrates that the partitioning of respiration between autotrophic and heterotrophic components has a significant impact on the conclusions drawn when comparing soil emissions across land-uses.

Figure 4.13a shows the total GHG measured using IRGAs/chambers with signifcantly higher fluxes observed in the grass land-uses when compared to the bioenergy crop, and only small changes observed between the arable and bioenergy crops. However, as stated previously, much of the CO_2 measured from grass sites evolves from above-ground respiration, which is not captured using the IRGA/chamber technique at the bioenergy and arable land-uses. Partitioning of respiration allows for comparisons of heterotrophic CO_2 production between the different land-uses, which gives an indication of how land-use change may be impacting microbial activity. Partitioning respiration is complex and although generalisations have been made in the literature about the relative contributions of auto- and heterotrophic respiration, these are likely to differ depending on soil conditions, productivity of the crop, life stage of the crop and arable crop type.

Figure 4.13b shows that GHG emissions (when using heterotrophic CO_2) are significantly lower in the majority of the bioenergy reference land-uses compared to the references. However, the direction of change is highly dependent upon how the respiration is partitioned between heterotrophic and autotrophic respiration, highlighting the need for further research in that area. In Section 4.4.4, the findings with regard to heterotrophic respiration are discussed in further detail.

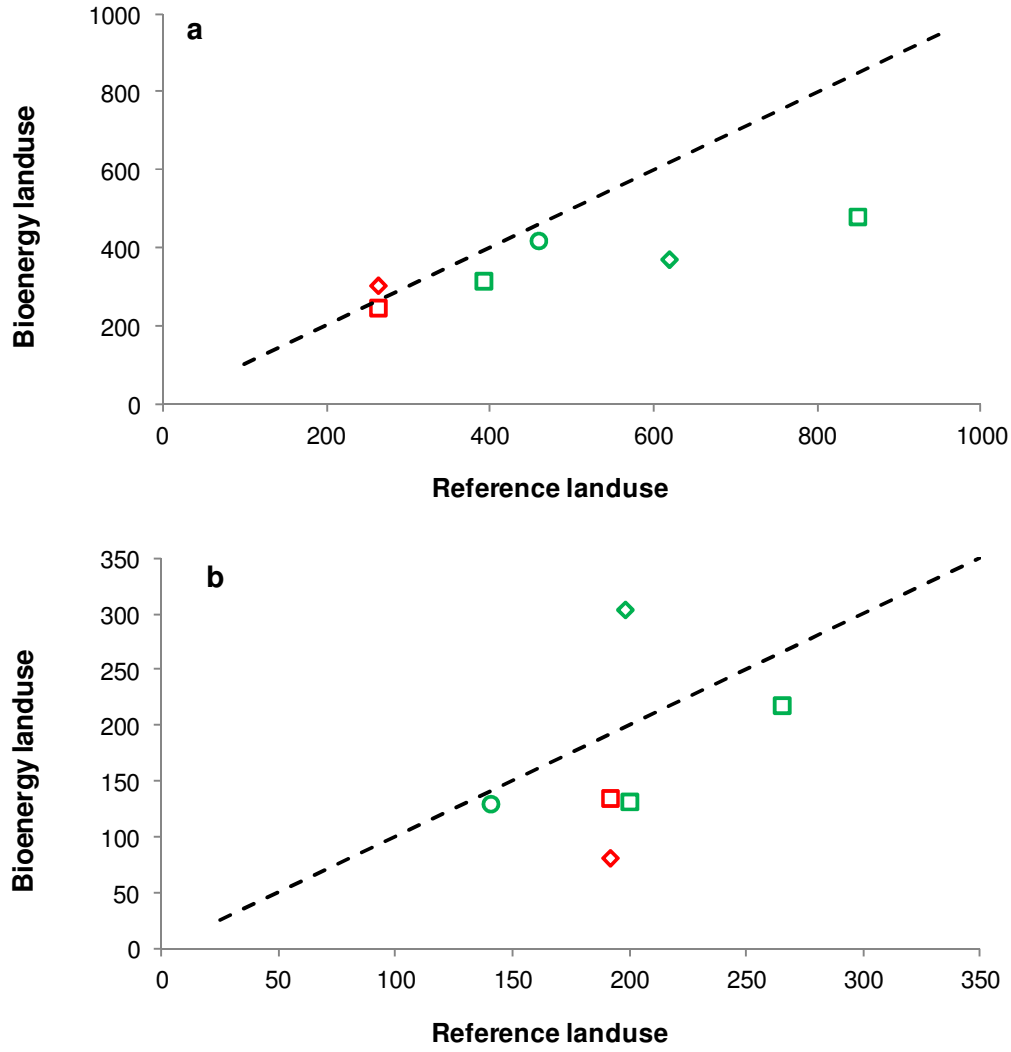

Figure 4.13: Fluxes of total GHG (CO_2, CH_4 and N_2O in mg-CO_2 eq. m^{-2} hr^{-1}) calculated using a) non-partitioned CO_2 flux (TER for grass and soil respiration for other land-uses) and b) partitioned heterotrophic respiration. Fluxes shown are an average over the two-year sampling period for the reference land-use (shown as green for grass and red for arable) and the bioenergy land-use (denoted by squares for *Miscanthus x giganteus,* circles for SRF, and diamonds for SRC willow). The 1:1 relationship is shown by the dashed line.

4.4.4. Heterotrophic respiration and soil GHG emissions

Overall, the largest changes in GHG fluxes were seen in the soil CO_2; however, these changes in heterotrophic respiration may be offset by changes in GPP and atmospheric CO_2 uptake. Changes in heterotrophic respiration following land-use change are of interest as microbial activity drives changes in soil C pools in models, but heterotrophic respiration alone cannot inform discussion regarding overall CO_2 losses and gains. NEE and overall GHG balances for the different land-uses are discussed in Sections 3 and 6 of this report.

Arable to bioenergy

Decreases in soil GHG emissions were observed with transitions from arable to bioenergy, with the lowest fluxes observed in the Lincolnshire and East Grange SRC willow sites. A note of caution is that this observation could relate to the partitioning factor applied to the

SRC willow for these two sites (heterotrophic respiration (R_h) is 25% of total respiration (R_{tot})), resulting in a large reduction from the measured flux in the SRC willow when partitioning is applied. However, the EC data from Section 3 demonstrate that for winter wheat (the arable crop in 2012), GPP was higher than for both the SRC willow and the *Miscanthus x giganteus* crop. Higher rates of root exudation associated with increased above-ground productivity could potentially drive higher heterotrophic CO_2 production in the arable crop.

Differences in management processes between the annual and perennial cropping systems can affect microbial activity and respiration. Increased microbial mineralisation of C has been associated with increased intensity of practices such as ploughing, fertilization and liming (Paustian *et al.*, 2000; Dawson & Smith, 2007). Land-uses that result in reductions in tillage are expected to have benefits for C storage (West & Marland, 2003), with decreases in litter decomposition expected to result from reduced disturbance from cultivation and management. In particular with SRC willow, changes in litter and root inputs result in changes in the soil fauna (Baum *et al.*, 2009). Stauffer *et al.* (2014) observed higher relative fungal abundance following a transition from arable to SRC which they suggest could relate to decreases in litter nutrient content and increases in lignin content. Fungal-dominated communities are associated with more efficient C and nutrient cycling (Bardgett & Wardle, 2010; de Vries & Bardgett, 2012; de Vries *et al.*, 2012), potentially resulting in lower C loss (as CO_2) from the litter and soils.

As mentioned in Section 3.2.3, EC measurements showed higher TER following the harrowing of the Lincolnshire *Miscanthus x giganteus* crop in April 2013. There was no significant difference in soil CO_2 flux observed with the IRGA/chamber-based method, although fluxes in the *Miscanthus x giganteus* were generally higher in 2013 compared to 2012. High within-field variability is likely to explain why no significant differences were observed in soil CO_2 fluxes between 2012 and 2013.

Grass to bioenergy

The conclusions to be drawn from grass transitions are less clear and there are confounding factors which should be highlighted when discussing these findings. There was little difference in soil GHG flux with the transition from grass to SRF at East Grange. It appears that SRF has little impact on overall soil GHG fluxes during the early years of establishment when the understory is undeveloped and still dominated by grass. It is likely that, as the SRF canopy develops and closure approaches, there will be a substantial change due the suppression of the grass sward.

In the genotype experiment at Aberystwyth, the reference grass plots were trimmed to reduce the contribution of grass respiration to the overall CO_2 flux. This is likely to have affected the below-ground activity, including increased homeostatic respiration from the grass roots, in response to above-ground damage. Source partitioning of respiration into below-ground auto- and heterotrophic respiration resulted in significantly lower fluxes of heterotrophic respiration from three of the *Miscanthus* genotypes (*Giganteus*, *Sin 1* and *Sin 2*) and the grass reference. This difference could result from a number of factors including the below-ground effects of 'weeding' the grass plots prior to measurement and the validity of <u>not</u> partitioning the CO_2 flux into above-ground grass respiration (potentially

resulting in the attribution of grass respiration to heterotrophic respiration). However, differences in C allocation below-ground between the *Miscanthus* genotypes and grass could drive changes in the cycling of plant-derived C. It has been shown that *Miscanthus* has high below-ground C allocation through deep roots, which may result in a greater proportion of plant-derived C becoming stabilised in the soil rather than being rapidly respired by heterotrophs near the soil surface (Anderson-Teixeira *et al.*, 2013).

It is important to note that at the Aberystwyth sub-sites A and B, a transition phase (from grass to *Miscanthus x giganteus)* is being monitored. Therefore, lower CO_2 fluxes observed in the *Miscanthus x giganteus* plots compared to the grass references may not represent what would be observed at a fully-established site. Assumptions made regarding the partitioning of respiration should also be applied with caution as autotrophic respiration is likely to contribute different proportions in the early stages of transition compared to once the crop is established. Statistics on partitioned heterotrophic respiration from the grass and the *Miscanthus x giganteus* post planting, show that there is not a significant release of heterotrophic derived CO_2 during the transition phase. Monthly measurements following conversion indicate that soil GHG emissions were significantly lower from the *Miscanthus x giganteus* plots compared to the grass.

The higher mean soil GHG emission observed from the grass to SRC willow transition at the West Sussex site is in contrast to the observations regarding SRC willow at the other network sites. This difference is likely to derive from the different partitioning proportions used for the West Sussex site (R_h=82% of R_{tot}) compared to the other two sites where SRC willow was measured (R_h=25% of R_{tot}); both values lie within the range reported in the literature (Hanson *et al.*, 2000). The West Sussex values were determined from field experiments run alongside the ELUM sampling whilst the values for the other sites have been drawn from the literature. This highlights the need for further data to be gathered regarding the partitioning of respiration in different crops as there is still great uncertainty in the contributions of autotrophic and heterotrophic respiration. Partitioning is clearly needed in the case of this work, as using measured CO_2 fluxes results in an unbalanced comparison between TER in the grass plots and soil respiration in the SRC willow plots.

5. ASSIMILATION OF CARBON BY BIOMASS CROPS

Summary

1. An in-situ ^{13}C pulse labelling approach during August 2012, was used, at the Lincolnshire Network site, to investigate C allocation and turnover in *Miscanthus x giganteus* and SRC willow.

2. A high rate of turnover of recently assimilated, pulse-derived C was found in leaf tissues of SRC willow and lower *Miscanthus x giganteus* leaves with 50% being lost within the first ~15 Hours after ^{13}C labelling. Upper *Miscanthus x giganteus* leaves exhibited a similar rate of decline until 48 hours post-pulse, when an increase in enrichment was observed indicating re-allocation of recent photosynthate to support new growth.

3. At 28 days post ^{13}C labelling, 62% of initially assimilated ^{13}C was retained in *Miscanthus x giganteus* upper leaves compared to 8% in the lower and SRC willow leaves while 24% and 39% of pulse-derived ^{13}C was retained in SRC willow and *Miscanthus x giganteus* stems respectively.

4. Initial, rapid losses can be attributed to a "fast" C pool with rapid turnover through leaf respiration and below-ground allocation in the form of soluble C compounds. The remaining fraction of ^{13}C was incorporated into a much slower turning over "Structural biomass pool" with C supporting growth and being locked into above and below-ground structural components or being re-allocated into short and long term storage.

5. In the case of *Miscanthus x giganteus*, a greater proportion of recently fixed ^{13}C appears to be retained within the "structural Biomass pool" relative to SRC willow which can partially be attributed to differences in growth phase between the two but may also indicate greater carbon use efficiency of *Miscanthus x giganteus*.

6. To achieve a more valid comparison between species at different stages of growth, a continuous labelling approach may be useful to estimate mean transfer rates through compartments and short term storage pools.

5.1 Introduction

Short term *in-situ* experiments concerning the fate of recently assimilated carbon (C) to the above- and below-ground components of leaf, stem, root and soil pools can yield valuable data required for predicting ecosystem C storage and turnover. Under land-use change, these pools are not at equilibrium and an important experimental challenge therein is to quantify the residence and trajectory of C in these pools. Many C allocation studies, that have focused on land-use change and management, have therefore taken advantage of ^{13}C pulse-chase studies (Ostle *et al.* 2000; Hogberg *et al.* 2008; Subke *et al.* 2009; Biasi *et al.* 2012). The short-term ^{13}C tracer approach does not override the utility of using long-term monitoring networks or space-for-time experiments (i.e. chronosequences); rather, it provides a new level of process understanding. The ^{13}C pulse-chase approach can provide valuable data for: C allocation to below-ground ecosystem components; the contribution of

photosynthate to heterotrophic and autotrophic fluxes; time lags between assimilation and soil respiration and the transfer of C to microbial and fungal pathways (Tavi *et al.* 2013). The most common field approach is through the exposure of plants to isotopically enriched ^{13}C in CO_2 at ambient (Ostle *et al.* 2000) or above-ambient concentrations (Hogberg *et al.* 2008) for several hours in clear chambers or tents. The photo-assimilation of $^{13}CO_2$ during this pulse labelling is then tracked through plant structures, bulk soil and into respiratory fluxes during the following days to months. The technique is referred to as the "$^{13}CO_2$ pulse-chase" approach due to the highly intensive nature of the field sampling that follows the isotope addition. This ^{13}C approach has been widely used for grassland and peatland ecosystems with shorter vegetation (Ostle *et al.* 2000; Carbone and Trumbore 2007; Ward *et al.* 2009; Subke *et al.* 2012; De Deyn *et al.* 2011). Recent ^{13}C pulse-chase experiments on whole tree (Hogberg *et al.* 2008; Subke *et al.* 2009; Epron *et al.* 2011; Kagawa, Sugimoto, and Maximov 2006) and large energy crop grass (Biasi *et al.* 2012) have demonstrated the potential for this technique at a larger scale but this is yet to be explored for second-generation bio-energy crops. The objective for this work was to make the first ever study of C storage and turnover of recently fixed CO_2, under co-located *Miscanthus x giganteus* and SRC willow fields at the Lincolnshire field site. The site description, crop cultivation and managements are described in Section 2 of this report.

5.2 Methods and Materials

5.2.1 $^{13}CO_2$ pulse labelling method

Our chamber design and ^{13}C pulse approach was similar to (Hogberg *et al.* 2008; Subke *et al.* 2009; Biasi *et al.* 2012). In each crop, 4 rectangular pulse chambers (6 m *l*, 2.5 m *w*, 3 m *h)* were erected resulting in a chamber volume of 45 m^3. This design allowed for the inclusion of two planted rows of willow SRC which were spaced 1.5 m apart whilst *Miscanthus x giganteus* was randomly distributed in the 15 m^2 area. Aluminium scaffold was used to support plastic polythene film which allowed 90% of photosynthetically active radiation (PAR) to enter the chamber. During the ^{13}C pulse, the chamber was sealed at the base, using a continuous line of sandbags on the tent skirt. In order to counter ambient air temperature increases within the chamber, each was cooled using 6.5 kW water cooled, split air conditioners capable of air movement of 1450 m^3/hr (Andrew Sykes, UK). In order to ensure adequate mixing of the label, additional air movement was facilitated by two tripod fans positioned at either side of the pulsing chamber. Eight individual petrol generators were used to provide power to each tent. During the ^{13}C pulse, air temperatures were recorded every three minutes inside and outside the tent, using Mini Nomad OM-80 Series temperature loggers (OMEGA Engineering inc.), so as to quantify the degree of cooling that was achieved. Plots from individual tents can be viewed in Appendix 1, Figure A1.3**.**

The ^{13}C pulse labelling was carried out on 23 August 2012 at *ca.* 08:20 hrs by introducing *ca.* 17 l of 99% ^{13}C-atom enriched pure CO_2 (CK Gases, UK) in sequential batches after sealing a tent. During the ^{13}C pulse, $\delta^{13}C$ isotopic delta values and total CO_2 concentration was monitored across all chambers using a G-2131i Series Isotopic CRDS (Cavity Ring Down) system (Picarro Inc, CA, USA) coupled to a multiplex, vacuum manifold, flow-through system fitted in a mobile laboratory (McNamara *et al.*, 2002). Ambient air from each chamber was delivered through PTFE sampling lines and flow was controlled through a system of flow

controllers and monitors maintaining a flow of 300 ml/min. The Multiplex system switched between tents every 3 minutes giving measurements approximately every 30 minutes per tent. During the pulsing period, this setup provided only an approximate estimate of the photosynthetic activity and enrichments achieved within the chambers, rather than absolute amounts as atom% levels of enrichment fall well outside the instruments dynamic range. After the tent sealing at approximately 07:00 hrs, we observed a rise in CO_2 concentrations followed by a decrease towards sub-ambient CO_2 concentrations, indicating photosynthetic activity outstripping ecosystem respiration. At this time the $^{13}CO_2$ was introduced in sequential batches over ca. 3 hours. CO_2 and $\delta^{13}C$ plots from individual tents can be viewed in Appendix 1, Figure A1.2.

5.2.2 Pulse-chase labelling

5.2.2.1 Gas sampling

Soil respiration gases were sampled one week prior to ^{13}C labelling and then at 4, 24 and 48 hours and then 4, 7, 14, 21, 28, 42, 76, 104 and 194 days after. The final sampling was made in March 2013. Sampling dates are summarised in Appendix 1, Table A1.1**.** Two PVC static chamber gas collars were permanently installed into the soil at equal spacing within the pulsed area to a depth of 2 cm below the surface, while one identical collar was positioned outside the experimental plot for periodic natural abundance control measurements. The chambers used were the same as described in Section 4.2.1 of this report. The chamber lid had a height of 20 cm and an internal diameter of 39 cm. When sealed with the chamber lid, the chambers (including 5 cm collar) had an internal volume of ~0.03 m^3 and a headspace volume of ~30 l. The chamber lids were covered with a reflective aluminium lid fitted with a pressure compensation valve and a central septum for gas collection with a needle and syringe. Headspace gas samples (20 ml, 0.066% of total chamber headspace volume) were taken using the static chamber method described by (Anthony, Hutchinson, and Livingston 1995) at 0, 15, 30 and 45 minutes post enclosure and injected into 12 ml gas-tight borosilicate glass vials (Labco, Lampeter, UK) for subsequent analysis. At each gas sampling, measurements of soil moisture, soil temperature and air temperature were made. Three soil moisture measurements were taken around each gas sampling chamber with a handheld ML2x Theta probe (Delta T Devices, Cambridge, UK) at a depth of 6 cm. Soil and air temperatures were taken at the beginning and end of each gas sampling around each chamber using a handheld temperature probe (Mini immersion thermometer, Testo Ltd, Alton, UK).

5.2.2.2 Plant material and soil collection

At each gas sampling event, solid samples of leaves, stems, roots and bulk soil were taken at each experimental plot across both *Miscanthus x giganteus* and SRC willow. Leaves and stems were taken from both upper and lower sections of plants across the plots. No SRC willow leaves were collected at 76, 104 and 194 days due to them being shed during senescence. Roots and soil samples were obtained with a 2.5 cm diameter gouge augur (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Three cores were taken and sectioned into 3 depths; 0-10, 10-20 and 20-30 cm. These were bulked in the field to give a total of three bulk roots and soil samples per experimental plot at 10 cm depth increments. For the *Miscanthus x giganteus,* rhizome samples were taken at 7, 14, 28, 42, 76, 104 and 194 days after pulse labelling. All solid samples were frozen at -23 °C as soon as possible

after collection. Vegetation and rhizome samples were cleaned and oven-dried at 60 °C followed by cryo-milling (SPEX SamplePrep, Freezer/Mill 6770) to a powder prior to analysis. Bulked soils were freeze-dried and then sieved to remove stones while coarse and fine roots were picked out and put into glass sample vials. The remaining soil was ball milled (Fritsch Planetary Mill Pulviresette 5) to a fine powder ready for analysis. Picked roots were oven-dried at 60 °C and cryo-milled.

5.2.2.3 Gas, soil and plant analysis

Gas samples were analysed separately for CO_2 concentration and $\delta^{13}C$ isotopic enrichment. 10 ml gas was removed from the glass sample vials via a syringe with a 2-way open/closed valve. These were attached to a 16-port distribution manifold feeding into a Small Sample Inlet Module (SSIM) and finally to a Picarro G-2131i CRDS (Cavity Ring Down) system where they were analysed automatically. A calibration gas sample (414 ppm, -9.98‰) was run after every sample. 5 ml of the remaining sample gas was transferred to a 3 ml borosilicate glass sample vial (Labco, Lampeter, UK) and run on a PerkinElmer Autosystem XL Gas Chromatograph (GC) (PerkinElmer, Waltham, MA, USA) fitted with a Flame Ionisation Detectors (FID) operating at 130 °C and Electron Capture Device (ECD) operating at 360 °C. The GC was fitted with a stainless steel Porapak Q 50-80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. 8 calibration gas standards (Air Products, Waltham on Thames, UK) were run per 32 samples and results were calibrated against these (Case *et al.* 2012).

Solid sample analysis was performed on a Costech ECS4010 Elemental Analyser (Costech Analytical Technologies Inc, CA, USA) coupled to a Picarro G-2131i CRDS analyser (Picarro Inc, CA, USA) via a split-flow interface using a method similar to (Balslev-Clausen *et al.* 2013). Samples were weighed into ultra-clean, 6 x 4 mm pressed tin cups (Elemental MicroAnalysis, UK), 2-3 mg for organic material and ~25 mg for bulk soils, crimped and loaded into a Zero N-Blank, 50 position carousel, autosampler. From the autosampler, samples were dropped at a throughput of 1 every 15 minutes into the combustion reactor. This was packed with Chromium Oxide (CuO_2) and Silvered Cobaltous Oxide (Co_3O_4/Ag) catalysts and maintained at a constant 980 °C. In the presence of pure oxygen, the tin capsule ignites and the contained sample thermally decomposes. Evolved CO_2 and nitrogen oxides are first passed through a reduction column, secondly passed through a GC column (HayeSep Q Porous Polymer, 3 m) for separation from other control gases, and finally through a Thermal Conductivity Detector (TCD) for C detection. Combustion gases were then vented through 1/16" Swagelok stainless steel tubing into the Picarro, Caddy split flow interface which matches flow rates, before passing into the Picarro CRDS analyser for $\delta^{13}C$ analysis. Standard materials covering a representative range of C and $\delta^{13}C$ values were run during each analysis batch and results were calibrated against these.

5.2.3 Calculations

5.2.3.1 Stable isotope notation

Studies of this kind have generally either expressed ^{13}C enrichment values in $\delta^{13}C$ (an isotopic signature, a measure of the ratio of ^{13}C and ^{12}C, reported in parts per thousand (‰) relative to a standard value (Pee Dee Belemnite - PDB)), or a ^{13}C atom% excess which expresses enrichment above a natural abundance background level. Outputs from the

Picarro $^{13}CO_2$ analyser were in standard delta (δ) value notation ($\delta^{13}C$) however all data was converted to the ^{13}C atom % excess form to conform to convention for samples enriched with ^{13}C as a tracer (Boutton, 1991) as numerous other studies of this kind have done (Leake *et al.* 2006; Ward *et al.* 2009; Kagawa, Sugimoto, and Maximov 2006). $\delta^{13}C$ values are calculated using the following equation:

Equation 1**:**

$$\delta^{13}C_{sample} = (((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{PDB}) -1)*1000$$

Where $^{13}C/^{12}C_{PDB}$ is the isotopic ratio of the standard material PDB given as 0.0112372. Results were converted to atom % values using the following equation:

Equation 2:

$$\text{Atom \%} = (100*AR*(\delta^{13}C/1000 +1)) / (1+AR*(\delta^{13}C/1000 +1))$$

Where AR = 0.011237. The absolute ratio of standard material (PDB) and $\delta^{13}C$ = standard delta value of sample.

Atom % is the absolute number of atoms of a given isotope in 100 atoms of an element (Ward *et al.* 2009). The ^{13}C excess represents enrichment above natural abundance background levels derived from the pre-pulse sampling of vegetation, root, rhizome and soil in the case of solid samples and headspace gas samples taken before the application of label. This is calculated using the following equation:

Equation 3:

$$^{13}C \text{ Atom \% excess} = \text{atom \%}_{\text{enriched sample}} - \text{atom \%}_{\text{background sample}}$$

5.2.3.2 Mass Balance and Flux Equations

Isotopic mass balance equations were used to calculate the amount of elevated or excess ^{13}C in soil respiration derived from the pulse.

Gas concentrations from the start and end of chamber closure (45 minutes) were then partitioned in to their ^{12}C and ^{13}C components using Equation 2. From this a ^{12}C and ^{13}C gas flux rate could be calculated using the following flux calculations (Equations 4 and 5):

Equation 4:

$$C_m = (C_v \times M \times P) / (R \times T)$$

Where C_m = Mass per volume concentration (µg CO_2-C / L), C_v = CO_2 concentration by volume (mixing ratio) (ppmv CO_2 - C), M = Molecular weight of CO_2, P = Barometric pressure (atm), R = Ideal gas constant defined as 0.08205746 L atm K^{-1} mol^{-1} and T = Air or chamber temperature at the time of sampling (K).

Flux is then calculated using:

Equation 5:
$F = (V \times C_{rate})/A$

Where F = Gas flux (mg CO_2 – C m^{-2} $h^{-1)}$, V = Internal volume of the enclosure (m^3), C_{rate} = Change in gas concentration over enclosure period (mg CO_2 m^3 h^{-1}) and A = area of collar enclosed soil surface (m^2).

The ^{13}C fluxes from the ^{13}C pulsed plots are a combination of pre-existing natural abundance ^{13}C (all environmental samples have background ^{13}C) and elevated pulse derived ^{13}C. To correct for the new and old ^{13}C, the amount ^{13}C in soil respiration in the absence of the pulse was calculated using chamber data from outside the ^{13}C pulsed plots. This was the Natural Abundance flux. The excess ^{13}C flux from the ^{13}C pulse was then calculated by:

Equation 6:
$^{13}C_{Excess}$ Flux (µg m^{-2} hr^{-1}) = $^{13}C_{Pulse\ labelling}$ flux – $^{13}C_{Natural\ Abundance}$ flux

5.2.4 Statistical methods

Comparisons of respiration fluxes and relative amounts of enrichment within different vegetation structures, positions and bulk C pools along with species comparisons were tested using the *nlme* linear mixed effects modelling package (Pinheiro and Chao 2006) within the R statistical software package (R Development Core Team, 2011). "Tent" was always included as a random effect to account for nested, repeated measures within *Miscanthus x giganteus* and SRC willow. For analysis of respiration fluxes, "chamber" was also specified as a random effect to account for the 2 sampling chambers nested within each experimental plot. The *Anova* function was used to interrogate fixed effects for significance. Where significance was found (Factors >2 levels), a Tukey, HSD Post Hoc test was performed using the *glht* general linear hypothesis test function in the *multcomp* package. Residuals were plotted to check for normality (Q-Q plot) and heteroscadicity, two key assumptions of ANOVA. Where variance was observed not to be heterogeneous, a weighting function was applied using the *varIdent* function.

5.3 Results and Discussion

5.3.1 Soil Respiration

Soil respiration rates for the SRC willow and *Miscanthus x giganteus* steadily declined as the growing season ended, in line with environmental conditions (Figure 5.1). Soil and air temperatures were the main drivers behind respiration rates with both having a highly significant positive effect *(ANOVA, p < .001***, p < .001*** respectively*). SRC willow respiration rates ranged from 122 to 5 mg CO_2-C m^{-2} hr^{-1} and *Miscanthus x giganteus* rates ranged from 101 to 7 mg CO_2-C m^{-2} hr^{-1} (Figure 5.2). Total soil respiration was significantly higher under SRC willow compared to *Miscanthus x giganteus (F = 22.20, p = .0033** One-way ANOVA).* This is unlikely to be due to environmental conditions as no significant differences between the two sites were seen (soil moisture (*$F_{1,6}$ = 0.0167 p = .9014), soil*

temp ($F_{1,6}$ = 0.00 p = .9641), air temp ($F_{1,6}$ = 2.473 p = .1669)) although all did change significantly over time (Table 5.1). This is not surprising, given the proximity and lack of any significant gradient between the two adjacent fields.

$\delta^{13}C$ values of soil respiration, measured from the two static chambers within the ^{13}C pulsed plots, were elevated above natural abundance levels in SRC willow and *Miscanthus x giganteus* (Appendix 1, Figure A1.1). The highest ^{13}C enrichments were observed during the first week at 48 hours following the ^{13}C pulse. As $\delta^{13}C$ values in respiration do not account for the quantity of carbon (i.e. a flux), only its ^{12}C:^{13}C isotopic ratio; isotope mass balance equations were applied to quantify the ^{13}C excess flux which was derived from the pulse. Results mirror the ^{12}C:^{13}C ratio data with greater pulse-derived ^{13}C flux from under the SRC willow plots compared to the *Miscanthus x giganteus* (Figure 5.2). ^{13}C excess in respiration was significantly higher for SRC willow compared to *Miscanthus x giganteus* ($F_{1,6}$ = 8.783, p = .0252*)(Table 5.3)) indicating a significantly higher rate of turnover of newly assimilated photosynthate, with the majority of ^{13}C labelled carbon appearing to be respired within the first 7 days (Figure 5.3).

Table 5.1: Summary statistics from LME model for differences in Moisture, Soil Temp and Air Temp between *Miscanthus x giganteus* and SRC willow over time

Environmental Parameter	Crop	Timepoint	Crop*Timepoint
Moisture	$F_{1,6} = 0.0167$ $p = .9014$	$F_{12,164} = 25.1145$ $p = <0.001^{***}$	$F_{12,164} = 3.8834$ $p = <0.001^{***}$
Soil Temp	$F_{1,6} = 0.00$ $p = .9641$	$F_{12,151} = 1348.50$ $p = <.001^{***}$	$F_{12,151} = 10.10$ $p = <0.001^{***}$
Air Temp	$F_{1,6} = 2.473$ $p = .1669$	$F_{12,149} = 514.137$ $p = <.001^{***}$	$F_{12,149} = 10.859$ $p = <.001^{***}$

Table 5.2: Summary Statistics from LME models on the effect of Crop, Moisture, Soil Temperature and Air temperature on CO_2 flux.

	Moisture	Soil Temp	Air Temp	Moisture* Soil Temp	Moisture* Air Temp	Crop
CO_2 Flux	$^1F_{1,165} = 0.94$	$F_{1,165} = 347.01$	$F_{1,163} = 184.53$	$F_{1,165} = 1.30$	$F_{1,163} = 0.58$	$F_{1,6} = 22.20$
	$^1p = .3338$	$p = <.001^{***}$	$p = <.001^{***}$	$p = 0.2568$	$p = 0.4456$	$p = .0033^{**}$
	$^2F_{1,163} = 4.12$	1 = Fixed effects of Soil Temp and Moisture				
	$^2p = 0.0439^*$	2 = Fixed effects of Air Temp and Moisture				

Table 5.3: Summary statistics from LME Model on the effect of crop on excess ^{13}C in soil respiration

	Crop
^{13}C Excess Flux	$F_{1,6} = 8.783$, $p = 0.0252^*$

Environmental Parameters

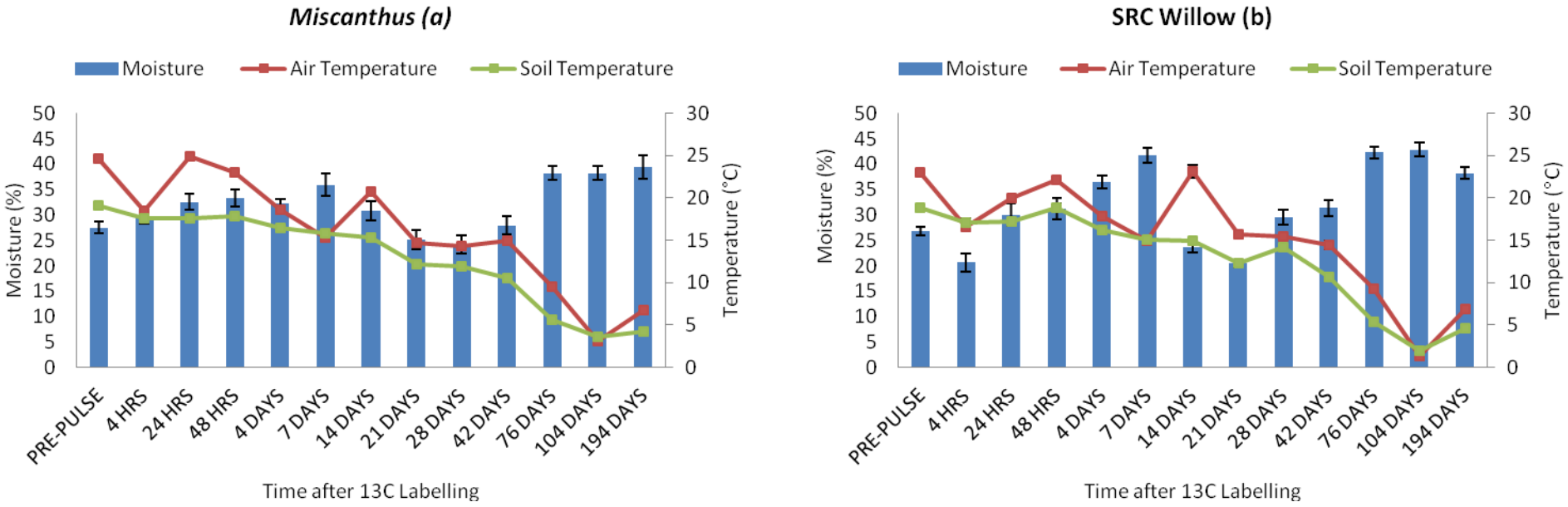

Figure 5.1: Moisture, Soil and Air Temperatures across *Miscanthus x giganteus* (a) and SRC willow (b) experimental plots from Pre-pulse to 194 Days after ^{13}C Pulse Labelling. Results are Means and error bars indicate standard errors for four replicate plots.

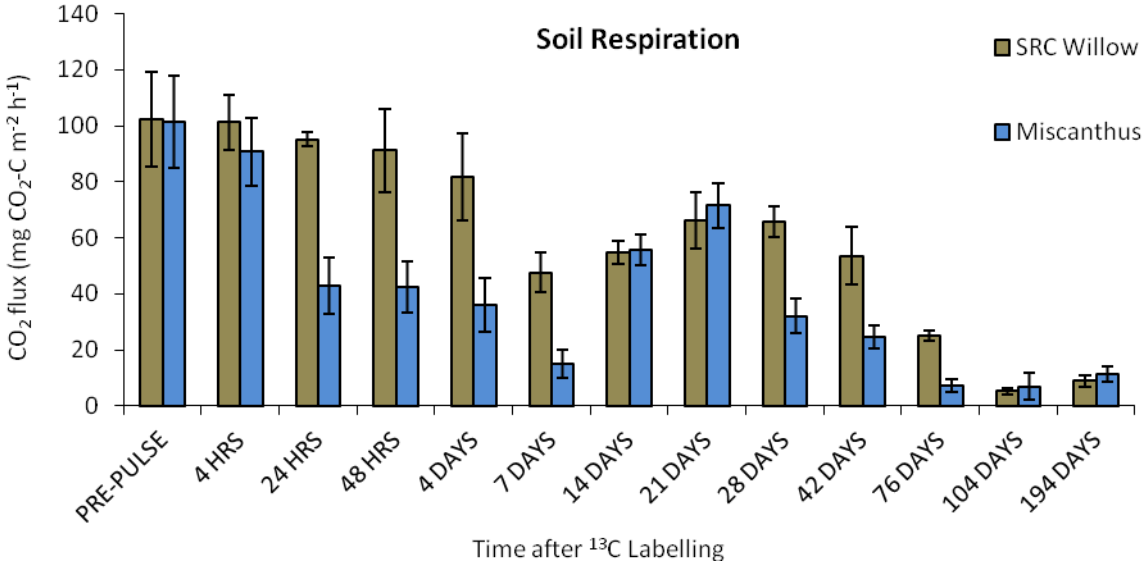

Figure 5.2: Soil respiration rates based on chamber data in mg CO_2-C $m^{-2}h^{-1}$ across *Miscanthus x giganteus* and SRC pulsed experimental plots from Pre-pulse to 194 Days after ^{13}C Pulse Labelling. Results are Means and error bars indicate Standard Errors ±1SE for four replicate plots.

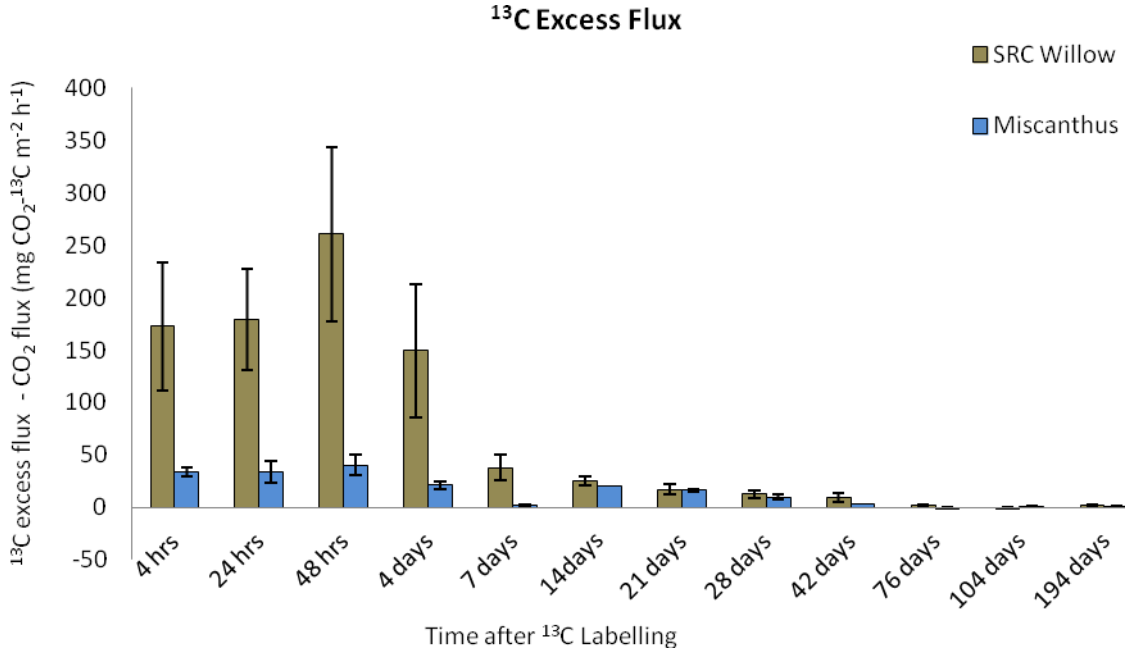

Figure 5.3: ^{13}C fluxes in excess of the natural background flux from SRC willow and *Miscanthus x giganteus*. These data represent C flux that has arisen from the recently fixed $^{13}CO_2$. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

5.3.2 Plant ^{13}C allocation

Natural abundance measurements, taken from vegetation collected before ^{13}C tracer addition, were typical of C3 plants (Boutton, 1991) at -28.56‰ and -29.66‰ for stems and leaves respectively. Enrichment within SRC willow leaves peaked at four hours following ^{13}C addition while stems peaked later at between ~24-48 hours post ^{13}C labelling highlighting the time lag between fixing of current photosynthate and subsequent transport into other plant structures (Figure 5.4). Significant differences in ^{13}C allocation were observed between positions and structures within SRC willow above-ground biomass. Significantly higher enrichments were recorded in upper positions ($F_{1,139}$ = 14.893, p = <.001***) and leaf structures ($F_{1,139}$ = 12.755, p = <.001***) relative to lower positions and stem structures respectively (Table 5.6). Rapid initial losses of newly-fixated carbon, were observed from the leaves. Leaves were collected up to 42 days post ^{13}C labelling at which point leaves were shed during senescence. Stems were collected for the final three samplings (76, 104 and 194 days) however statistics were run on the timeseries up to 42 days after ^{13}C labelling.

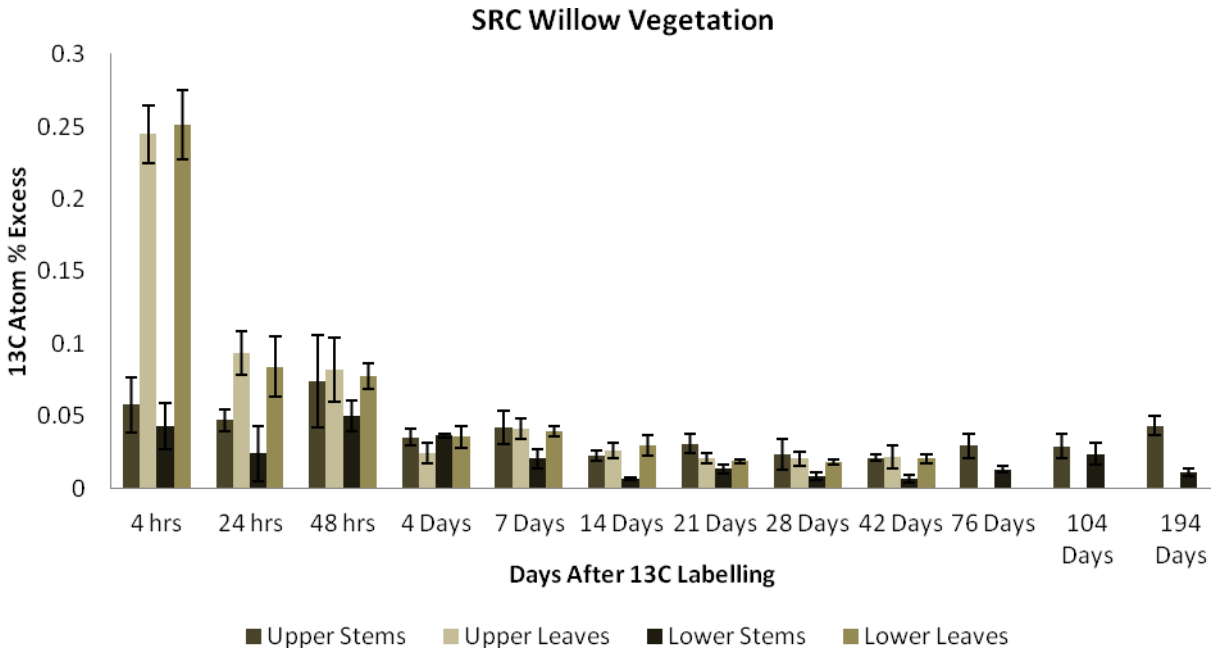

Figure 5.4: ^{13}C atom % Enrichment plotted as an excess over natural abundance for SRC willow leaf and stem structures from Pre-pulse to 194 Days after ^{13}C Labelling. These data represent newly fixed labelled photosynthate into plant structures and its rate and fate over time. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

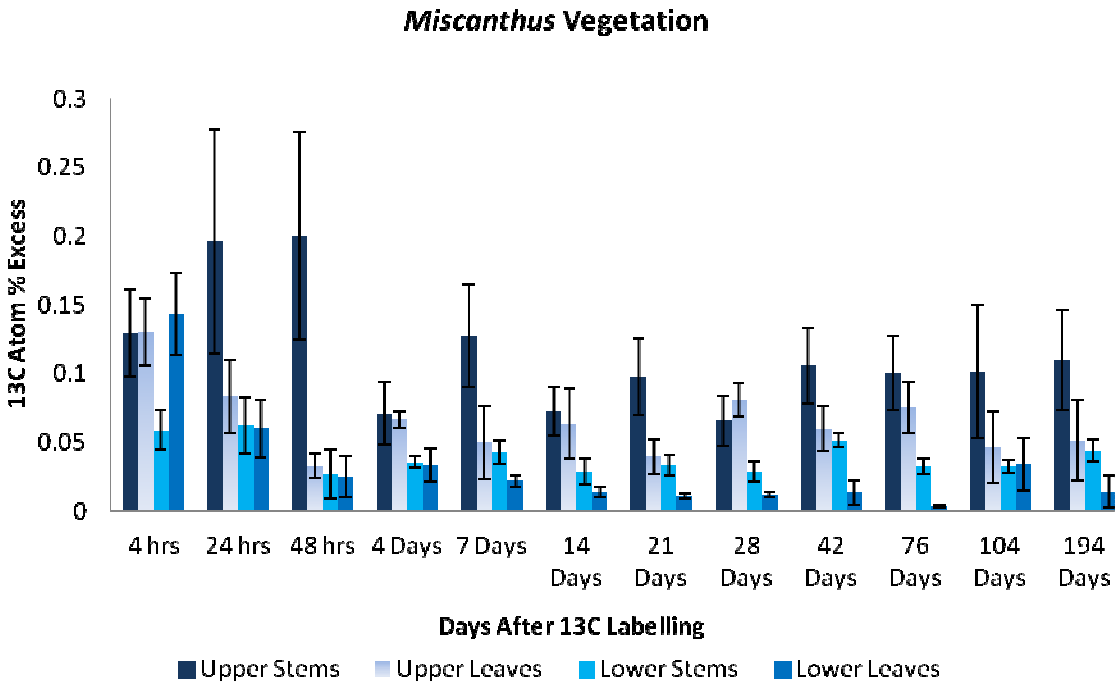

Figure 5.5: ^{13}C atom % Enrichment plotted as an excess over natural abundance for *Miscanthus x giganteus* leaf and stem structures from Pre-pulse to 194 Days after ^{13}C Labelling. These data represent newly fixed labelled photosynthate into plant structures and its rate and fate over time. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

Natural Abundance measurements made before the application of ^{13}C were typical of C4 plants (Boutton, 1991) at -11.81 ‰ & -11.84 ‰ for stems & leaves respectively. Enrichment within *Miscanthusx giganteus* leaves peaked at 4 hours after ^{13}C labelling while stem enrichment peaked later at between ~24-48 hours post ^{13}C labelling mirroring trends observed within SRC Willow (Figure 5.5). Significant differences in ^{13}C allocation were observed between positions and structures within *Miscanthus x giganteus* aboveground biomass (whole time-series). Greater levels of ^{13}C enrichment were measured in upper positions ($F_{1,138}$ = 69.925, p = <.001***) and stem structures ($F_{1,138}$ = 14.158, p = <.001***) relative to lower positions and leaf structures respectively (Table 5.6). There was no overall difference in enrichment over the total chase period between *Miscanthus x giganteus* and SRC Willow ($F_{1,6}$ = 1.875, p = .220) (Table 5.4) however labelled ^{13}C was distributed differently in the two crops. SRC Willow was more enriched in leaves ($F_{1,6}$ = 7.748, p = <.05*) but less enriched in stems relative to *Miscanthus x giganteus* ($F_{1,6}$ = 19.253, p = <.01**) while upper structures (Leaves & Stems) were as a whole more enriched in *Miscanthus x giganteus* ($F_{1,6}$ = 19.253, p = <.01**) due to the apparent greater retention of labelled ^{13}C within actively growing stem structures (Table 5.5). There was no significant difference in enrichment within lower structures.

Table 5.4: Statistics for difference in enrichment between SRC Willow and *Miscanthus x giganteus* over time.

	Crop	Timepoint	Crop*Timepoint
Enrichment	$F_{1,6}$ = 1.875, p = .220	$F_{8,263}$ = 13.209, p = <.001***	$F_{8,263}$ = 1.927, p = .056

Table 5.5: Statistics for differences in distribution of labelled C between SRC Willow & *Miscanthus x giganteus*.

	Crop
Upper	$F_{1,6}$ = 19.253, p = <.01**
Lower	$F_{1,6}$ = 1.055, p = .34
Stems	$F_{1,6}$ = 19.253, p = <.01**
Leaves	$F_{1,6}$ = 7.748, p = <.05*

Table 5.6: Significances for SRC willow and *Miscanthus x giganteus* Positions and Structures over 42 Days. One-way ANOVA with tent as random effect to account for repeated measures.

Vegetation	Position	Structure
SRC willow ^{13}C enrichment	$F_{1,139}$ = 14.893, p = <.001***	$F_{1,139}$ = 12.755, p = <.001***
Miscanthus x giganteus ^{13}C enrichment	$F_{1,138}$ = 69.925, p = <.001***	$F_{1,138}$ = 14.158, p = <.001***

5.3.3 Roots ^{13}C allocation

Enrichment within SRC willow roots appeared to follow a positive trend over time possibly indicating an accumulation of ^{13}C (Figure 5.6). Root samples were also sectioned into three depths (0-10 cm, 10-20 cm, 20-30 cm). No significant differences were observed between them ($F_{2,125}$ = 0.603, p = 0.549) indicating a relatively homogenous delivery of pulse-derived C to root structures.

^{13}C enrichment within *Miscanthus x giganteus* roots appeared to follow a positive trend over time, similar to SRC willow indicating an accumulation of pulse derived C (Figure 5.7). Root samples were sectioned into three depths (0-10 cm, 10-20 cm, 20-30 cm) but no significant differences in ^{13}C enrichment were observed between them ($F_{2,134}$ = 1.049, p = .353) (Table 5.7). Rhizomes were collected at selected timepoints throughout the chase period (Figure 5.8) which seem to mirror root enrichment until 194 days after ^{13}C labelling. At this point rhizomes appear to lose pulse derived ^{13}C. This could be down to the translocation of unlabelled C to the rhizomes, in the form of nutrients, as stems and leaves die off during the winter.

Table 5.7: Statistics for the effects of depth on ^{13}C root enrichment under SRC willow and *Miscanthus x giganteus*

Roots	Depth
SRC willow ^{13}C enrichment	$F_{2,125}$ = 0.603, p = 0.549
Miscanthus x giganteus ^{13}C enrichment	$F_{2,134}$ = 1.049, p = 0.353

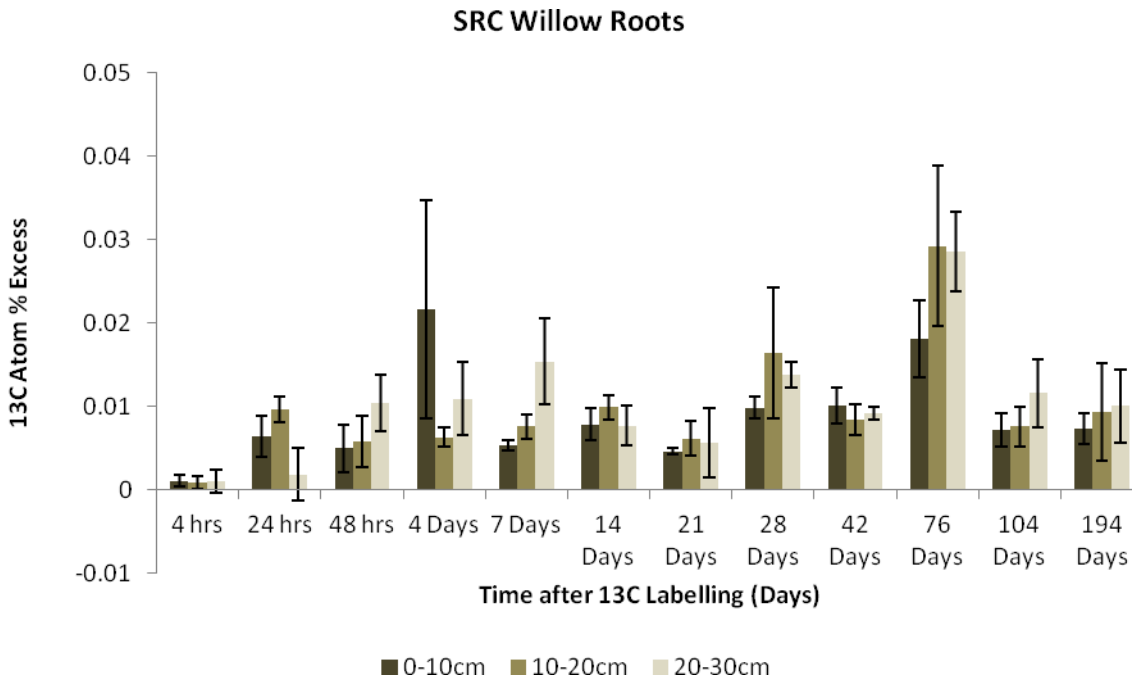

Figure 5.6: ^{13}C atom % Enrichment plotted as an excess over natural abundance for SRC willow roots from Pre-pulse to 194 Days after ^{13}C Labelling. Roots were sectioned into three depths of 10 cm. These data represent recently fixed labelled photosynthate transported from above-ground vegetation to root structures over time. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

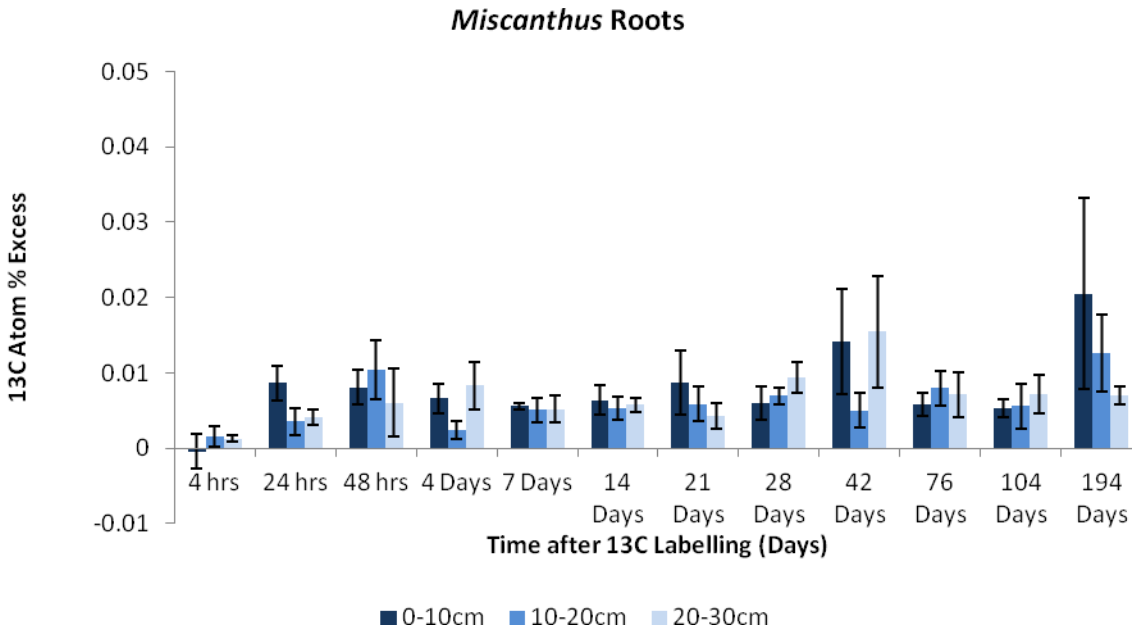

Figure 5.7: ^{13}C atom % Enrichment plotted as an excess over natural abundance for *Miscanthus x giganteus* roots from Pre-pulse to 194 Days after ^{13}C Labelling. These data represent recently fixed labelled photosynthate transported from above-ground vegetation to root structures over time. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

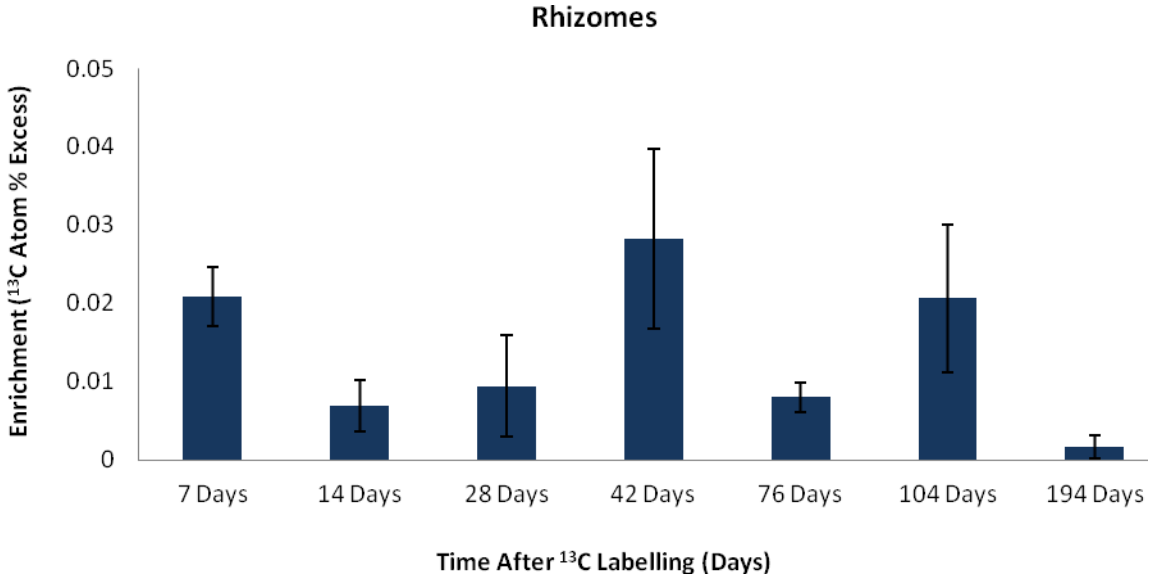

Figure 5.8: ^{13}C atom % Enrichment plotted as an excess over natural abundance for *Miscanthus x giganteus* rhizomes from 7 Days to 194 Days after ^{13}C Labelling. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

5.3.4 Soil ^{13}C allocation

Enrichment in the SRC willow bulk soil pool was generally low and variable. This was not unexpected. The majority of fixed pulse-derived ^{13}C appears to be quickly lost through plant and soil respiration. Only a very small proportion is made available to be added to the total soil C pool which is very large in comparison. However significant differences between depth sections were observed ($F_{2,138}$ = 6.332, p = 0.002**) (Table 5.8). A Post-Hoc Tukey HSD Test performed on depth showed significantly greater enrichment in the lower 20-30 cm section relative to the top 0-10 cm section. Differences between other depth horizons were non-significant (Table 5.9).

Enrichment in the *Miscanthus x giganteus* bulk soil pool was again low and variable most likely due to the reasons outlined above. Significant differences between depth sections were observed ($F_{2,138}$ = 23.660, p = <.0001***) (Table 5.8). A Post-Hoc Tukey HSD Test was performed on depth which showed significantly greater enrichment in the top 0-10 cm section relative to both deeper sections (10-20 cm and 20-30 cm) (Table 5.9).

Table 5.8: Statistics for the effects of depth on ^{13}C bulk soil enrichment under SRC willow and *Miscanthus x giganteus*

Bulk Soil	Depth
SRC willow ^{13}C enrichment	$F_{2,138}$ = 6.332, p = 0.002**
Miscanthus x giganteus ^{13}C enrichment	$F_{2,138}$ = 23.660, p = <0.0001***

Table 5.9: Post-Hoc Tukey HSD test on depth as a significant factor for bulk soil ^{13}C enrichment under SRC willow and *Miscanthus x giganteus*

Crop	Depth	Estimate	Std Error	p value
SRC willow	10-20 cm-0-10 cm	0.0004	0.0002	0.099
	20-30 cm-0-10 cm	0.0007	0.0002	0.0011**
	20-30 cm-10-20 cm	0.0003	0.0002	0.300
Miscanthus x giganteus	10-20 cm-0-10 cm	-0.0014	0.0002	<0.001***
	20-30 cm-0-10 cm	-0.0007	0.0002	0.0013**
	20-30 cm-10-20 cm	0.0007	0.0002	0.0021**

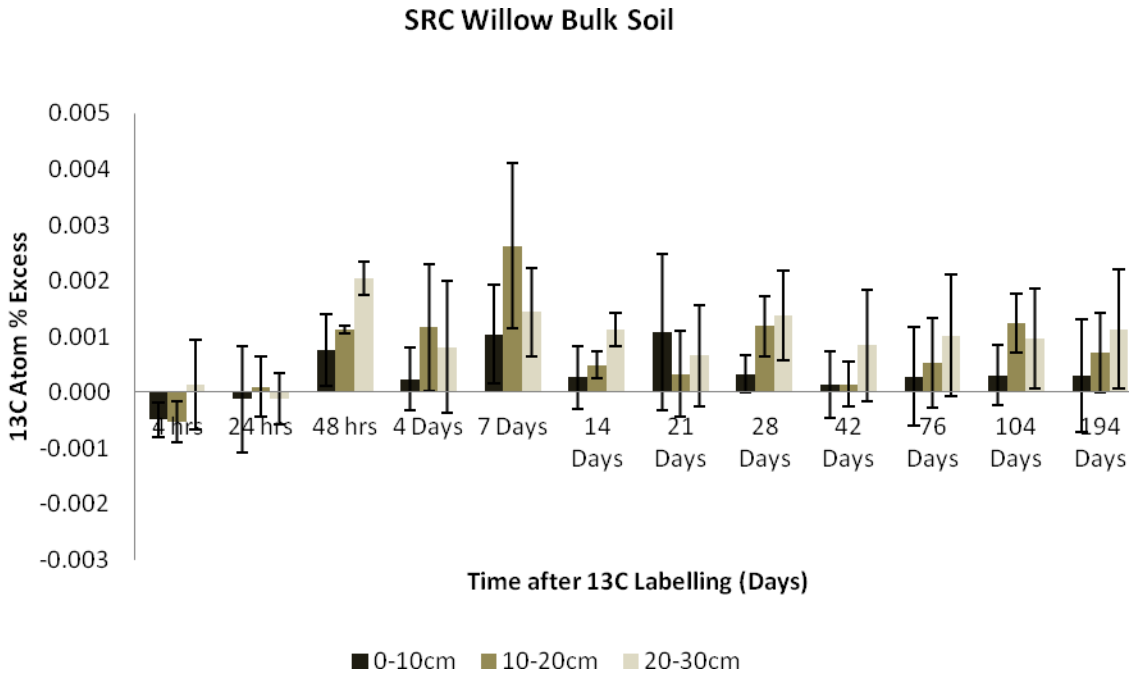

Figure 5.9: ^{13}C atom % Enrichment plotted as an excess over natural abundance for SRC willow bulk soil from Pre-pulse to 194 Days after ^{13}C Labelling. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

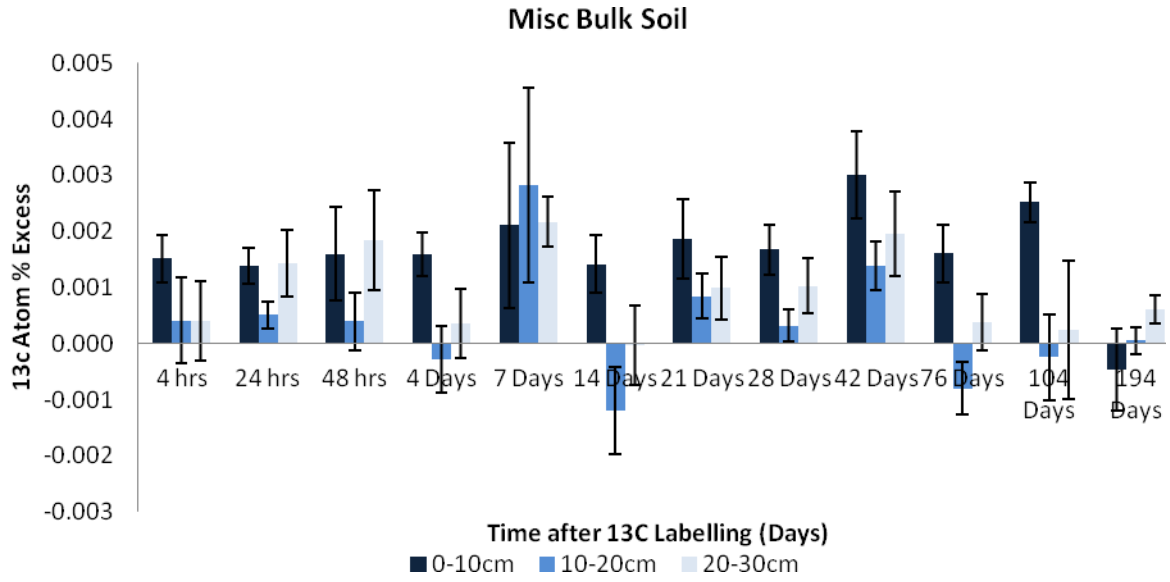

Figure 5.10: ^{13}C atom % Enrichment plotted as an excess over natural abundance for *Miscanthus x giganteus* bulk soil from Pre-pulse to 194 Days after ^{13}C Labelling. Results are Means and error bars indicate Standard Errors ±1 std err for four replicate plots.

5.4 Carbon Pool ^{13}C Allocation

To consider ^{13}C transport and accumulation within each distinct pool, simplification of the data was necessary. Upper and lower leaves and stems were grouped together to make a total above-ground vegetation pool while below ground, roots from the 3 depth sections (0-10 cm, 10-20 cm, 20-30 cm) were also grouped together. The same procedure was carried out for bulk soil giving three carbon pools; vegetation, bulk roots and bulk soil. This enabled the comparison of accumulation within each. In the case of *Miscanthus x giganteus*, a fourth Rhizome pool was added. Rhizomes were taken at eight timepoints during the chase period; 7, 14, 28, 42, 76, 104 and 194 days.

5.4.1 SRC willow

SRC willow vegetation had significantly higher enrichment over the first 42 Days relative to Bulk Roots and Bulk Soil. Bulk Roots had significantly greater enrichment relative to Bulk Soil (Table 5.10-5.11) indicating greatest C allocation above-ground.

Table 5.10: Significances for SRC willow C Pools over 42 Days. One-way ANOVA with tent/time as random effect to account for repeated measures over time.

SRC willow	F-value	p-value
CPool	205.523	<.001***

Table 5.11: Post Hoc Tukey LSD Test on C Pool as a significant factor

CPool	Estimate	Std Error	p-value
Soil - Roots	-0.006	0.0005	<.001***
Vegetation - Roots	0.015	0.0014	<.001***
Vegetation - Soil	0.022	0.0013	<.001***

5.4.2 Miscanthus x giganteus

Miscanthus x giganteus vegetation had significantly higher enrichment, considering the entire chase period of 194 days, relative to Rhizomes, Bulk Roots and Bulk Soil. Bulk Roots were significantly more enriched relative to Bulk Soil but no significant difference was observed between roots and Rhizomes. (Table 5.12-5.13).

Table 5.12: Significances for *Misc* C Pools over 8 time-points. One-way ANOVA with tent/time as random effect to account for repeated measures over time.

Miscanthus x giganteus	**F-value**	**p-value**
CPool	102.042	<.001***

Table 5.13: Post Hoc Tukey LSD Test on C Pool as a significant factor

CPool	Estimate	Std Error	p-value
Roots - Rhizome	-0.0013	0.0014	0.747
Soil - Rhizome	-0.0069	0.0013	<.001***
Vegetation - Rhizome	0.0433	0.0045	<.001***
Soil - Roots	-0.0056	0.0005	<.001***
Vegetation - Roots	-0.0446	0.0043	<.001***
Vegetation - Soil	0.0502	0.0043	<.001***

5.5 SRC Willow – *Miscanthus x giganteus* comparisons

5.5.1 Vegetation

Although each crop was at a different growth phase, comparisons were made between SRC willow and *Miscanthus x giganteus* ^{13}C allocation in above-ground vegetation pools (Upper and Lower Stems and Leaves) and between total C pools as described below in Section 5.5.2.

Stem enrichments were significantly higher in *Miscanthus x giganteus* relative to willow over the entire chase period (One-Way ANOVA, p-.009**) while in the leaves, higher ^{13}C enrichment was observed in the SRC willow (One-Way ANOVA p-.0318**). Higher enrichments were observed in the Upper section of the plant (Stem and Leaf combined) for *Miscanthus x giganteus* relative to willow (One-Way ANOVA p-0.0095**) while no significant differences were seen in the lower section.

5.5.2 Carbon pool allocation

Comparisons were also made between overall C pools. Overall *Miscanthus x giganteus* retained more labelled photosynthate within its above-ground vegetation structures than SRC willow when considering the whole time series (p-.0024**), however no significant differences were observed in the bulk roots and bulk soil pools.

5.5.3 Vegetation C residence times

In order to assess differences in C turnover rates, we considered a simplified C-transfer model with two general pools of carbon: a “fast, labile pool” and a “structural biomass pool” (Subke *et al.* 2012). During the ^{13}C pulse period, a high proportion of CO_2 fixed by the plants was in the form $^{13}CO_2$ due to the artificially-induced, localised enrichment above natural abundance. Not all C, fixed by the plants during the pulse, was ^{13}C as some ^{12}C remained within the tent along with a constant addition from plant and soil respiration during the enclosure period. Initially, all new labelled photosynthate formed part of the “fast labile C pool”. This can be respired, form temporary storage in the form of soluble carbohydrates, used for new growth or allocated to below-ground pools. The remaining ^{13}C becomes incorporated into various structures of the plant and forms a “structural biomass pool”. In an attempt to quantify the rate of turnover of the labile C pool, data was fitted to an appropriate mathematical function which yielded the best fit to the data. Previous studies have used both exponential and logarithmic functions to model decay rates following ^{13}C or ^{14}C pulse addition (Leake *et al.* 2006; Subke *et al.* 2012). Data was fitted to exponential, logarithmic and power functions. In this case, a power function was found to best describe the post ^{13}C labelling decline. This is a power-law relationship where one variable varies as a power of another. Power curves were fitted from the time of peak enrichment (4 hours in leaves, 24-48 hours for stems) observed to 28 days after ^{13}C labelling under the assumption that this time period would best capture the majority of turnover of labile C. This fits the observations seen in ^{13}C excess flux from below-ground respiration (Figure 5.3).

Power functions follow the general form:

Equation 7:
$F(x) = ax^b$

Where parameter a is a simple scaling factor which has the effect of moving the values of x^b up or down as a increases or decreases respectively. b represents the exponent which controls the functions rate. Positive exponents represent growth while negative represents decay. Decay rates can then be calculated by solving the function. The non-zero asymptotes of the fitted curves represent the slower structural biomass pool.

Key assumptions considered when modelling ^{13}C decay are that the measured reduction in ^{13}C enrichment was due solely to losses via leaf respiration, phloem transport to other structures of the plant or incorporation into structural carbon (Biomass pool). In reality, some dilution will have occured due to the fixation of unlabelled CO_2 after the ^{13}C pulse.

Estimated half-lives (when y=50% of maximum enrichment) for labile C were calculated for both *Miscanthus x giganteus* and SRC willow Leaves and Stems at Upper and Lower positions of the plant. SRC willow estimated half-lives were 0.62 and between 5.30-7.55 days for leaves and stems respectively. *Miscanthus x giganteus* estimated half-lives were between 0.61-3.10 for leaves and 7.84-15.99 for stems.

5.5 Discussion

The aim of this study was to investigate the rate of C turnover and storage, by using the ^{13}C tracer technique, on 2nd generation bio-energy crops, SRC willow and *Miscanthus x giganteus* for the first time. Using gas flux measurements and solid sample analysis of vegetation, root and soil material, we were able to quantify rates of C turnover through leaves and stems and compare overall C dynamics between the two species.

Differences were observed between rates of turnover of assimilated ^{13}C as evidenced by significantly higher ^{13}C excess fluxes in soil respiration below the SRC willow and relative quantities of retained ^{13}C in leaf and stem tissues. A large proportion of new photosynthate formed from pulse-derived ^{13}C was lost extremely rapidly from leaves. 50% was lost in just 0.61-0.62 days (~15 Hours) in all SRC willow and lower *Miscanthus x giganteus* leaves (Figures 5.11 and 5.12). This was partly due to plant respiration during the first night after ^{13}C labelling, but also to rapid below-ground allocation and turnover. This is evidenced by high ^{13}C excess fluxes, especially under the SRC willow where fluxes were significantly higher during the first few days after labelling. Upper *Miscanthus x giganteus* leaves showed a more complex dynamic with similar loss rates up to 48 hours after ^{13}C labelling followed by a rise in ^{13}C enrichment and subsequent stabilisation. This may indicate preferential allocation of resources to upper leaves to support new growth.

The above-ground carbon dynamics of both species can be summarised as a simple two-pool system; a “fast labile C pool” and a slower turning “structural biomass pool”. The steep initial decline in ^{13}C enrichment, shown in Figures 5.11 and 5.12 by the fitted curves, represents losses from the “fast” pool with the majority of this being lost through respiration and re-allocation within the first seven days. The non-zero asymptotes represent the smaller “structural biomass” pool formed from retained ^{13}C. Overall, *Miscanthus x giganteus* appears to retain a greater proportion of assimilated ^{13}C within its structures as indicated by significantly lower ^{13}C excess in soil respiration throughout the pulse-labelling period, particularly within the first seven days. 28 days after labelling SRC willow retained ~24% of initial maximum ^{13}C enrichment in stems and ~8% in leaves while *Miscanthus x giganteus* retained ~39% and ~35% in stems and leaves respectively. There was a large difference between *Miscanthus x giganteus* upper and lower leaves with 62% and 8% of initial ^{13}C label retained respectively. This represents more active growth and C allocation into structural components in the upper portion of the plants. This greater retention of C in above-ground *Miscanthus x giganteus* biomass suggests a higher overall carbon-use efficiency with respect to SRC willow. However, one must consider that the two crops were at different

phases of their respective growth cycles. Peak Gross Primary Productivity (GPP) and Net Ecosystem Exchange (NEE) rates were observed during early June to late July for SRC willow, whereas *Miscanthus x giganteus* Peak GPP and NEE rates coincided with the ^{13}C pulse labelling between late July and early September (see Section 3.3). Higher variability in ^{13}C fixation within *Miscanthus x giganteus* relative to SRC willow may be attributable to this.

Variability observed between all replicate plots may have been caused by the relative amounts of $^{13}CO_2$ initially fixed, which controls to a large degree, the magnitude of allocation of labelled ^{13}C to below-ground root and soil pools (Leake et al, 2006). During this experiment, remote monitoring of air within the tents indicated up to 40 atom % enrichment levels shortly after ^{13}C additions. These levels of enrichment were indicative only, as the instrument was operating far outside its calibrated range. However it is important to note that ^{13}C added was constantly being diluted by unlabelled ecosystem respiration from plants and soil. In addition, the age and position of leaves may have also played a part in the amount of $^{13}CO_2$ ultimately fixed into plant tissues.

Roots represented the second, most enriched C pool after above-ground vegetation. An apparent accumulation of ^{13}C was observed in both *Miscanthus x giganteus* and SRC willow roots suggesting some structural C allocation. Conclusions however, must be drawn with caution as the discrimination between live and dead roots in bulk soil is difficult. A strategy to avoid this uncertainty may be to harvest roots directly from plants rather than picking from bulk soil samples, however, this method would be more destructive. ^{13}C enrichment within *Miscanthus x giganteus* rhizomes closely followed those observed in roots until the final sampling of the chase period on 5th March (194 days). An increase in root ^{13}C and a decrease in rhizome ^{13}C may indicate possible re-allocation of C from temporary storage within the rhizomes to the roots as the new growing season approached.

Conclusions from bulk soil measurements are difficult to draw as ^{13}C enrichment was slight and variability was high between replicates. This was not entirely unexpected as the addition of ^{13}C was small in comparison to the size of the bulk soil C pool. Furthermore, much of this ^{13}C is unavailable as it is respired or structurally allocated. A compounding factor beneath the *Miscanthus x giganteus* may be heterogeneity in the distribution of older C derived from previously grown C3 crops (oil seed rape, winter wheat) and newer C derived from the *Miscanthus x giganteus (C4)* itself. C3 plants generally have a $\delta^{13}C$ of ~ -27‰ to -29‰ while C4 plants have $\delta^{13}C$ isotopic signature of ca. -12‰ (Schneckenberger and Kuzyakov 2007). Some significant differences in ^{13}C enrichment between depths were observed. Significantly higher ^{13}C enrichment at 20-30 cm under SRC willow indicates a greater root density, relative to shallower horizons, supplying ^{13}C in the form of root exudates (rhizodeposition). Under the *Miscanthus x giganteus*, significantly higher ^{13}C enrichment was observed in the upper 0-10 cm section of bulk soil. This is surprising given the deep rooting structure of *Miscanthus x giganteus* plants (Drewer *et al.* 2012).

Soil and microbial respiration were the main loss mechanisms for the majority of recent photosynthate directed below-ground into bulk soil via rhizodeposition. This was shown by peak ^{13}C enrichments in soil respiration only 48 hours after pulse. However it is important to note that a significant fraction of ^{13}C excess flux measured during the first days post-labelling may be derived from direct diffusion from the soil following exposure to atom % enrichment

levels (Ostle *et al.* 2000; Leake *et al.* 2006). However, the amount of ^{13}C returned follows the total rates of soil respiration, strongly suggests that this is a biotic response. The fact that SRC willow and *Miscanthus x giganteus* crops were at different growth phases during ^{13}C labelling complicates the process of drawing direct comparisons of carbon dynamics between the two. However, the magnitude of the differences seen, particularly in ^{13}C fluxes from soil respiration indicate fundamental differences in the rate, efficiency and ultimately fate of fixated carbon. This may be linked to the fact that *Miscanthus x giganteus* is a C4 plant which fixes more CO_2 per unit of water and nitrogen than do C3 plants such as SRC willow or that, unlike *Miscanthus x giganteus;* SRC willow roots can be associated with not only arbuscular but also ectomycorrhizal fungi (Hrynkiewicz *et al.* 2010) which may facilitate more rapid turnover of labile C supplied below-ground.

This pulse labelling has been a technical challenge in terms of the sheer scale of the field event and with respect to laboratory developments regarding isotopic analysis of gas and solid samples. Key learning points to consider are that to achieve a more valid comparison between species with different growth patterns, multiple ^{13}C pulses or a continuous labelling approach across a longer time period would be useful to gain greater understanding of the magnitude of seasonal and growth stage impacts on C fixation into plants and soils. Short ^{13}C pulses are useful for assessing maximum transfer velocity from leaves to other compartments as labile C becomes much more strongly labelled than structural compounds (Hanson *et al.* 2000), while a continuous labelling approach could prove useful in investigating mean transfer rates through compartments including short term storage pools (Studer, Siegwolf, and Abiven 2014).

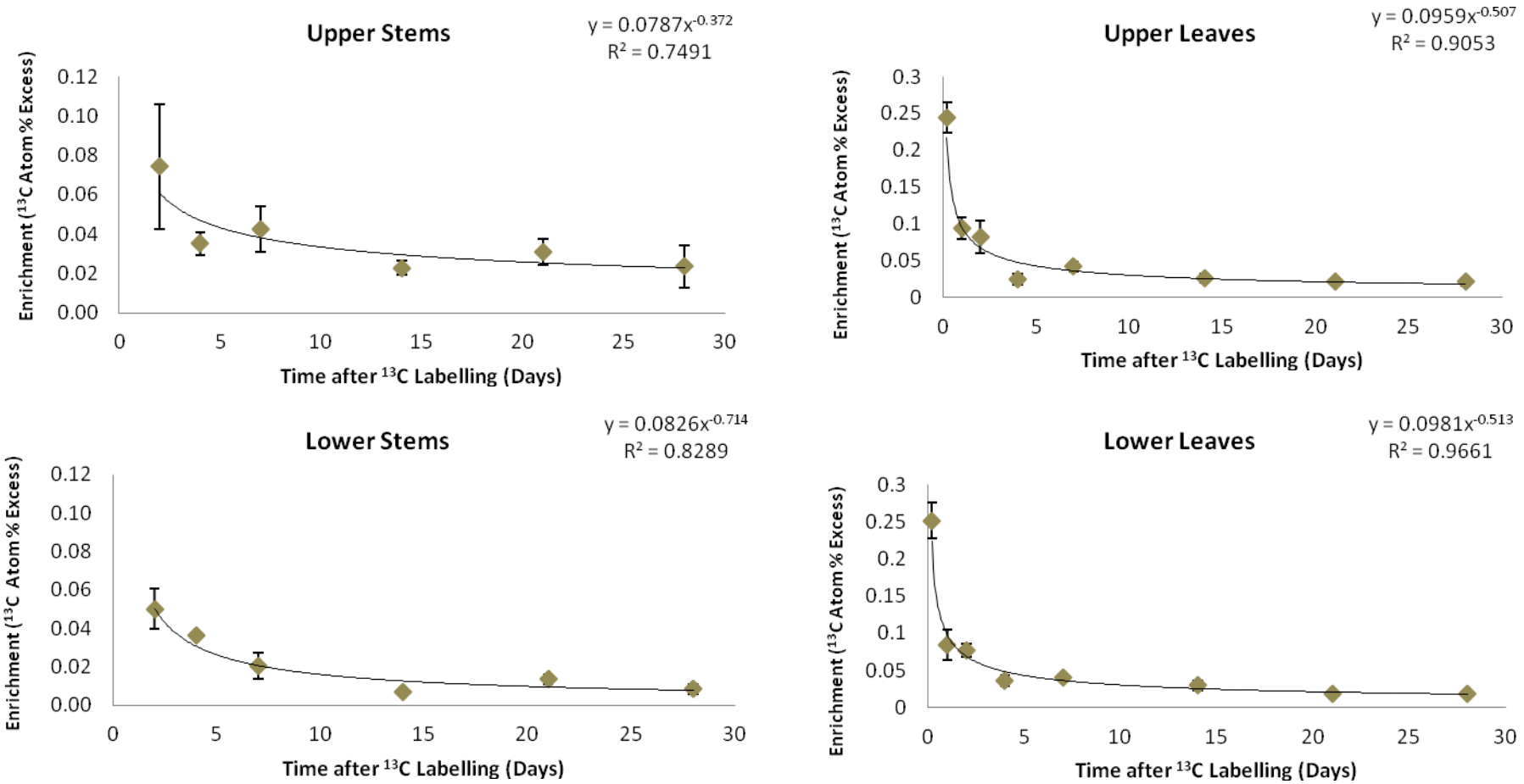

Figure 5.11: ^{13}C atom % excess enrichment in SRC willow stems and leaves up to 28 days post ^{13}C labelling. Results are means and error bars represent ±1SE for four replicate plots. Lines are fitted power curves for means. Note different Y-axis between plots.

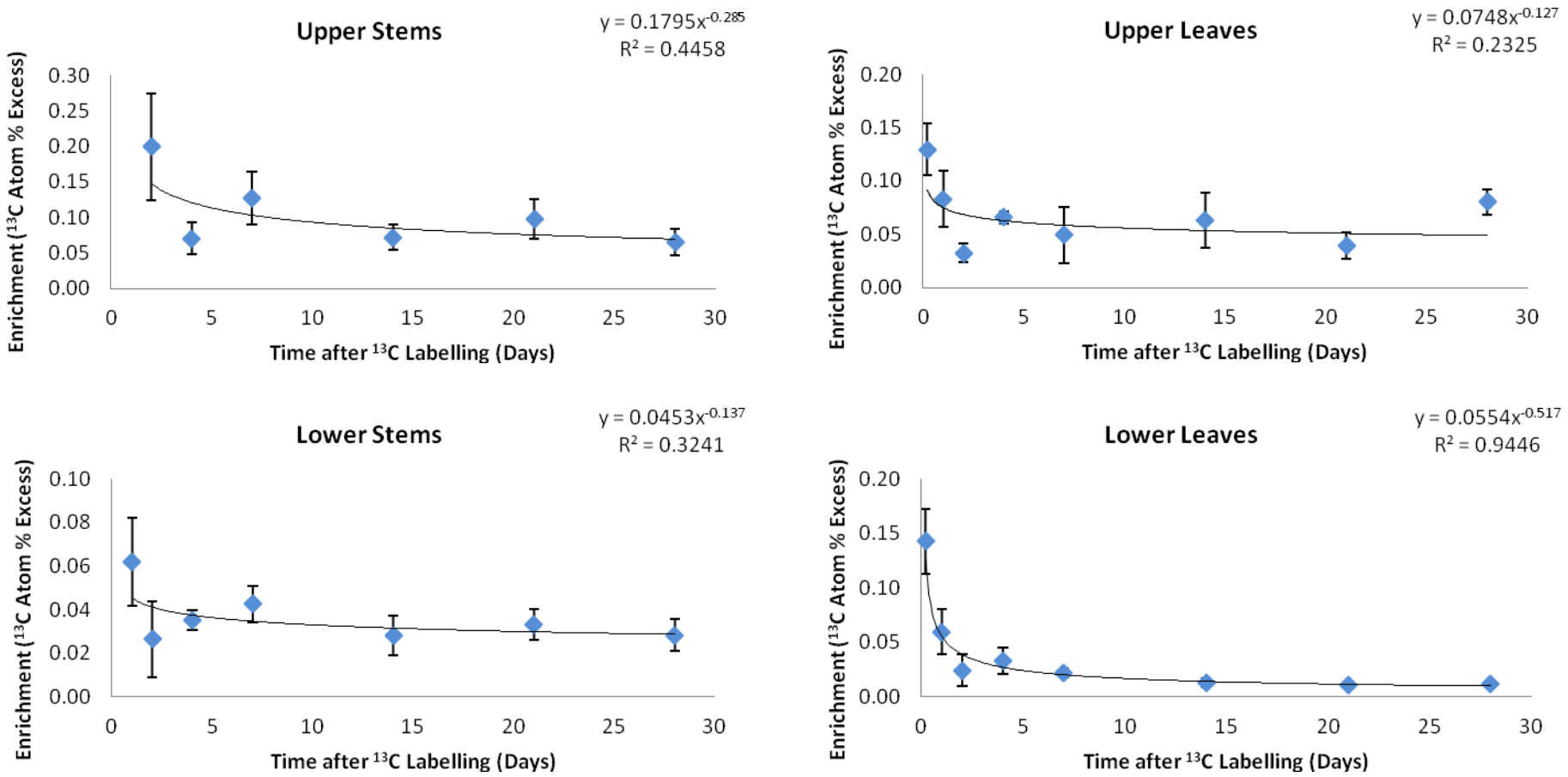

Figure 5.12: ^{13}C atom % excess enrichment in *Miscanthus x giganteus* stems and leaves up to 28 days post ^{13}C labelling. Results are means and error bars represent ±1SE for four replicate plots. Lines are fitted power curves for means. Note different Y-axis between plots.

Table 5.14: Summary table of Maximum enrichment, time to reach 50% enrichment and remaining enrichment after 28 days in SRC willow stems and leaves.

Structure	Max Enrichment (Days)	Max Enrich (^{13}C Atom % Excess)	50% Enrichment (Days)	Enrichment Remaining at 28 Days (%)
Upper Stems	2.0	0.074	7.55	31.70
Upper Leaves	0.2	0.245	0.62	8.51
Lower Stems	2.0	0.050	5.30	16.69
Lower Leaves	0.2	0.251	0.62	7.39

Table 5.15: Summary table of Maximum enrichment, time to reach 50% enrichment and remaining enrichment after 28 days in *Miscanthus x giganteus* stems and leaves.

Structure	Max Enrichment (Days)	Max Enrich (^{13}C Atom % Excess)	50% Enrichment (Days)	Enrichment Remaining at 28 Days (%)
Upper Stems	2.0	0.200	7.84	32.7
Upper Leaves	0.2	0.130	3.10	62.1
Lower Stems	1.0	0.062	15.99	45.8
Lower Leaves	0.2	0.143	0.61	8.1

6. CARBON ALLOCATION AND TURNOVER IN THREE *MISCANTHUS* GENOTYPES – A COMPARISON

SUMMARY

1. An in-situ ^{13}C pulse labelling approach was used during July 2013, at Aberystwyth sub-site C, to investigate C allocation and turnover in three *Miscanthus* genotypes; *Miscanthus x giganteus (Giganteus)*, *Miscanthus sinensis (Sinensis)* & *Miscanthus sacchariflorus/lutarioparius (Sac/Lut).*
2. There were no significant differences detected in total soil respiration or ^{13}C excess values in respiration between the genotypes. This largely reflects what was observed during the two years of soil respiration measurements at this site.
3. There was a small time-lag of approximately 1 day evident between ^{13}C fixed in the top leaves of the plant compared to the leaves and stems in the rest of the plant.
4. The *Sac/Lut* had significantly less enrichment of ^{13}C in vegetation than the other two genotypes, with 50% of peak enrichment values lost in the top leaves after 2 days and in the rest of the plant after 4 days. On day 130, *Sac/Lut* had only 5.4% enrichment left in its leaves & stems, while the the *Giganteus* and *Sinensis* had 13.3% and 18.3% left respectively.
5. As rhizomes were observed to decrease in ^{13}C enrichment, the corresponding roots gained ^{13}C suggesting re-allocation of C between these plant components. The ^{13}C enrichment levels in roots were significantly lower than in the rhizomes for all genotypes.
6. From above-ground vegetation to rhizome to root and into the soil, the level of^{13}C enrichment decreased in all genotypes.
7. The main differences between genotype with regard to carbon pool allocation was that *Sac/Lut* was less enriched in ^{13}C in above-ground biomass than the other two genotypes and *Sinensis* was more enriched in the roots.
8. An aim of *Miscanthus* breeding programmes is to increase genetic diversity and increase tolerance traits in future cultivars. However, an evaluation of commercial varieties with future varieties and under different environmental conditions would be prudent. This research indicates that although some differences were evident in C pool allocation and time, above ground morphological differences (including yield, *Giganteous>Sac/Lut>Sinensis*) did not impact on C stock and soil ^{13}C respiration rates.
9. For all genotypes, the majority of fixed, pulse-derived ^{13}C enrichment in the soil pool was quickly lost through plant and soil respiration with only a very small proportion made available to be added to the soil C pool.

6.1 Introduction

Research across Europe has predominantly focused on one single clone, *Miscanthus x giganteus (Giganteus)* which is a sterile, triploid interspecific hybrid (Clifton-Brown*et al*., 2000). Research with *Giganteus* has demonstrated the potential of the crop throughout Europe, and within the range of genotypes currently available*, Giganteus* was the most productive in much of Northern Europe (Farrell*et al*., 2006). The *Giganteus* genotype has been chosen for widespread use for commercial planting, however, it is costly to establish as it is clonally propagated by rhizomes. A major breeding target is to introduce genetic

diversity to the crop through the development of novel, high-yielding cultivars. By way of contrast, in plantations of SRC willow, up to six genotypes are planted together to help mitigate the impact of pest attack. To date there has been no research into the C sequestration potential of different *Miscanthus* varieties. *M.sinensis (Sinensis)* is commercially available but is predominantly used as an ornamental plant rather than as an energy crop. Research has shown that it is not as vulnerable as *Giganteus*, with regard to late spring frosts(Clifton-Brown *et al*., 2004) and has a higher combustion quality than *Giganteus* (Lewandowski *et al*., 1997). The *M.sacchariflorus/lutarioparius* (*Sac/Lut*) has demonstrated the ability to produce high yields during field trials, while at the same time showing considerable variation in the response of yield to different site conditions (Clifton-Brown *et al*., 2000). Figure 6.1 depicts the above ground morphological differences and yield data from 2012/2013 collected at the Aberystwyth site.

Giganteus	Sinensis	Sacchariflorus/Lutarioparis
Yield 2013: 16 t/h ±2	Yield 2013: 6 t/h ±1	Yield 2013: 11 t/h ±1.5
Maximum canopy height 2013: 310 cm ±6	Maximum canopy height 2013: 200 cm ±4	Maximum canopy height 2013: 380 cm ±4
Total leaf fall 2012/13: 911 g/m^2 ±210	Total leaf fall 2012/13: 580 g/m^2 ±78	Total leaf fall 2012/13: 795 g/m^2 ±85
Leaf decomposition over 1 year: 58 % mass loss ±6	Leaf decomposition over 1 year: 46 % mass loss ±9	Leaf decomposition over 1 year: 46 % mass loss ±2

Figure 6.1: The three genotypes. The *Giganteus* and *Sinensis* as individual plants were all easily identified as they grew in bunches from the rhizome, while the *Sac/Lut* rhizomes spread below ground and individual plants were difficult to decipher. These pictures were taken on the day of the ^{13}C pulse.

Due to the morphological above-ground differences between the genotypes, it was hypothesised that carbon pool allocation, amounts and transfer time would also have been different. The objective for this work was to undertake the first ever study of C storage and turnover of recently fixed CO_2 under three different *Miscanthus* genotypes to determine whether these hypothesised differences were present.

6.2 Materials & Methods

6.2.1 Field description

This trial was planted in 2010 with 25 m^2 plots, with each genotype planted in triplicate and distributed randomly across the field (Figure 6.2).

Figure 6.2: Aerial photo taken in July 2011 illustrating the genotype distribution

6.2.2 $^{13}CO_2$ pulse labelling method

The chamber design and ^{13}C pulse approach was similar to that described in Chapter 5, section 5.2.1. In each replicate plot, ^{13}C pulse-chase tents (2m *l*, 2m *w*, 3m *h*) covered with transparent film were erected, resulting in a tent volume of 12 m^3(Figure 6.1). The tent material allowed 90% of photosynthetically active radiation (PAR) to enter the tent. During the ^{13}C pulse, the tent was sealed at the base, using a continuous line of sandbags on the tent skirt. In order to help control tent warming, each tent was cooled using a split air conditioner**.** Additional air movement was facilitated by a tripod fan positioned at the alternate side of the tent as the air conditioner unit. In contrast to the experimental work undertaken in 2012 (section 5.2.1), a single diesel generator provided power to all tents, rather than having one generator per tent.

The ^{13}C pulse labelling was carried out on 26thJuly 2013 at *ca.* 08:20 hours by introducing *ca.*6 l of 99% ^{13}C-atom enriched pure CO_2 in sequential batches after sealing the tent (CK Gases, UK). During the ^{13}C pulse, 20ml gas samples were frequently taken by syringe and stored in 12 ml gas-tight exetainer vials (Labco, Lampeter, UK) for subsequent ^{13}C and CO_2 concentration analyses (see Chapter 5, section 5.2.1).

6.2.2.1 Gas sampling

Soil ^{13}C-CO_2 flux measurements were made one week prior to ^{13}C labelling and then after labelling at 4, 24, 48 hours followed by less frequent sampling on days 3, 4, 5, 7, 10, 14, 28, 56, 84 and 130. The final gas sampling day was in December 2013. Sampling dates are

summarised in the table A2.1 (Appendix 2). For gas measurements, two PVC static chamber gas collars (15 cm *d*, 10 cm *h*) were permanently installed into the soil at random spacing within the ^{13}C pulsed area to a depth of ~2 cm. Results from the chambers were pooled. When the chamber lid (15 cm *d*, 20cm*h*)was sealed into the collars, the overall headspace volume was 0.005 m^3. The chamber lids had a central septum for gas collection with a needle and syringe. Headspace gas samples (20 ml, 0.4% of headspace volume) were taken using the static chamber method outlined in section 4.2.1 at 0, 15, 30 and 45 minutes post enclosure and injected into12ml exetainer vials for subsequent analysis. Gas ^{13}C processing, analysis and the statistical handling of results are as in Chapter 5, section 5.2.

On each gas sampling day, measurements of soil moisture, soil temperature and air temperature were taken. Three soil moisture measurements were taken in each plot with a handheld ML2x Theta probe (Delta T Devices, Cambridge, UK) at a depth of 6 cm. Soil and air temperatures were taken at the beginning and end of each gas sampling around each chamber using a handheld temperature probe (Mini immersion thermometer, Testo Ltd, Alton, UK).

6.2.2.2 Plant Material and soil collection

At each gas sampling event (except 48 hrs, days 4, 5 and 10, but with an extra sampling on day 190 post-pulse) solid samples of leaves, stems, roots, rhizomes and bulk soil were taken at each experimental plot. Leaves were taken from the upper-most part of the plant with the rest of the leaves and stems bulked together as one sample. Only three plots out of nine had top leaves available for sampling on day 190 due to senescence: one *Giganteus* plot and two *Sinensis* plots. Roots and rhizomes were taken by digging with a shovel near the base of a randomly selected plant in the tented area and soil samples were obtained with a 2.5cm diameter gouge augur (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Three cores were taken and sectioned into 3 depths; 0-10, 10-20 and 20-30 cm. These were later bulked in the field to give a composite sample for each layer of each plot. All solid samples were frozen at -23°C after collection and then freeze-dried. Vegetation, root and rhizome samples were cryo-milled (SPEX SamplePrep, Freezer/Mill 6770) to a powder prior to ^{13}C analysis. Soils were sieved to remove stones and fine roots and then ball-milled (Fritsch Planetary Mill Pulviresette 5) to a fine powder ready for analysis. Bulk sample ^{13}C analysis, processing and the statistical handling of results are as in Chapter 5.2.

6.3 Results & Discussion

6.3.1 Soil Respiration

There were no significant differences between genotypes with regard to the environmental conditions, but as expected, there were significant differences with time (Table 6.1). Soil respiration rates for all genotypes steadily declined as the growing season ended, in line with environmental conditions (Figure 6.3). Soil and air temperatures were the main drivers behind soil respiration rates with both having a highly significant positive effect (ANOVA, $p < 0.001$, $p < 0.001$ respectively). Moisture was seen to have a significant negative effect, which is to be expected as in general, as temperatures increase, soil moisture levels decrease. There were some differences evident between genotypes with regard to soil respiration (Figure 6.4) at 7 to 14 days post-labelling, with *Sac/Lut* appearing to respire more

than the other two genotypes. Overall, however there were no significant differences in total soil respiration rates between the genotypes (Table 6.2).

^{13}C values of soil respiration, measured from the two static chambers within the ^{13}C pulsed plots, were elevated above natural abundance levels in each of the plots. The highest ^{13}C enrichments (Figure 6.5) were observed during the first week at 24 hours following the ^{13}C pulse in all three genotypes, it then decreased rapidly in the following 24 hours, decreasing slowly until day 7, and after much lower ^{13}C effluxes were observed. As ^{13}C values in respiration do not account for the quantity of carbon (i.e. a flux) – only its ^{12}C:^{13}C isotopic ratio – isotope mass balance equations were applied to quantify the ^{13}C excess flux which was derived from the pulse. Although there were differences observed at the earlier time points – in particular for *Giganteus* which appeared to have a lower level of enrichment – overall there were no significant differences in total ^{13}C excess between genotypes (Table 6.3).

Table 6.1: Summary statistics from LME model for differences in soil moisture, soil temperature and air temperature in all three genotypes over time.

Environmental parameter	Time-point
Soil moisture	$F_{1,197}$=27.47 p=<0.0001
Soil temperature	$F_{1,197}$=75.6 p=<0.0001
Air temperature	$F_{1,197}$=41.4 p=<0.0001

Table 6.2: Summary statistics from LME models on the effect of crop, soil moisture, soil temperature and air temperature on soil CO_2 flux.

	Soil moisture	Soil temperature	Air temperature	Genotype
CO_2 flux	$F_{1,199}$=107.8 p=<0.0001	$F_{1,199}$=115.7 p=<0.0001	$F_{1,199}$=20.92 p=<0.0001	$F_{2,6}$=0.653 p=0.55

Table 6.3: Summary statistics from LME model on the effect of genotype on excess ^{13}C in soil respiration.

	Genotype
^{13}C Excess flux	$F_{2,6}$=2.84 p=0.14

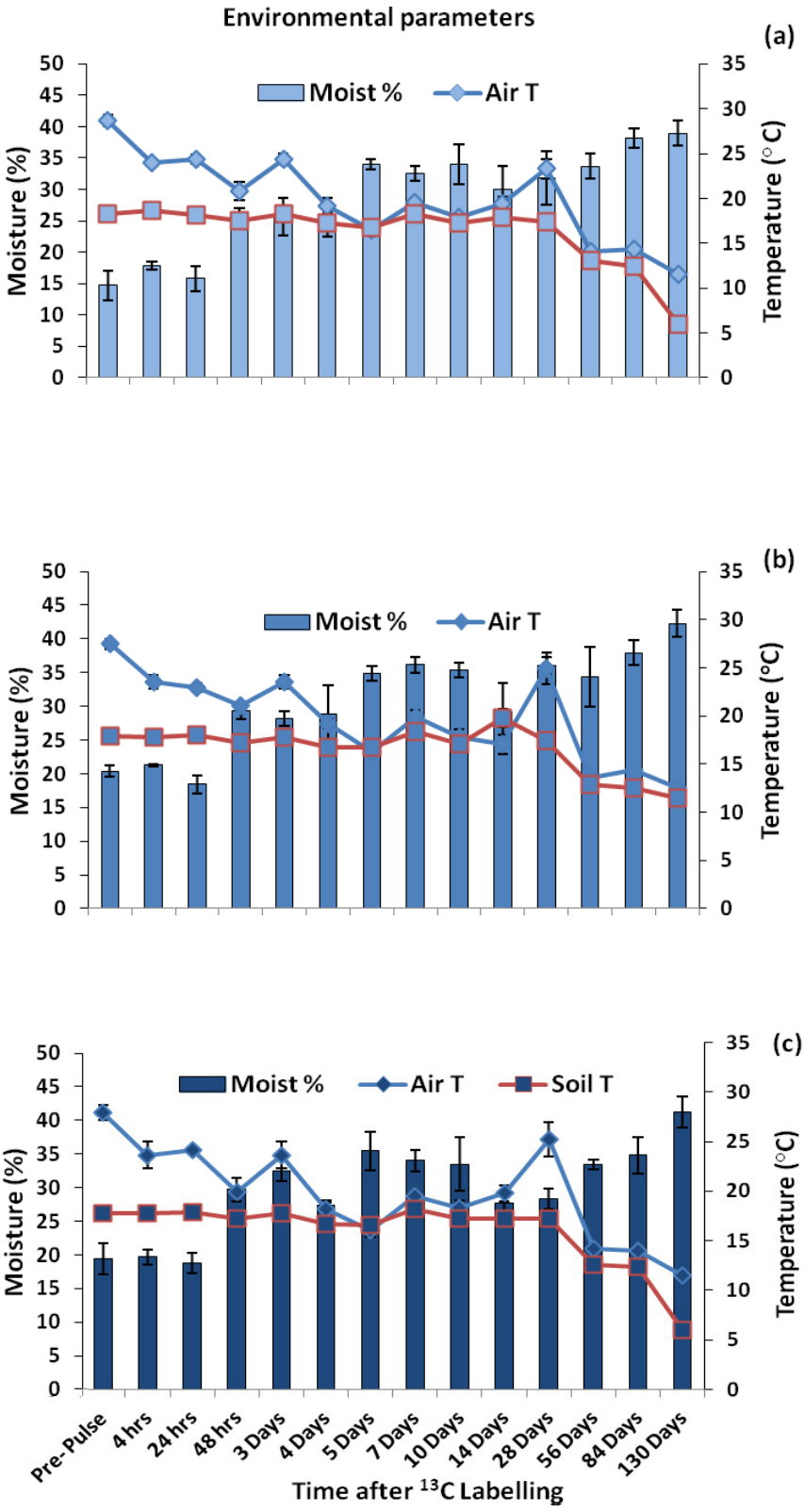

Figure 6.3: Environmental parameters; soil moisture content, soil temperature and air temperature (a) *Sac/Lut,* (b) *Sinensis* and (c) *Giganteus* from pre-pulse up to 130 days after the pulse. Results are means and standard errors for the three replicate plots.

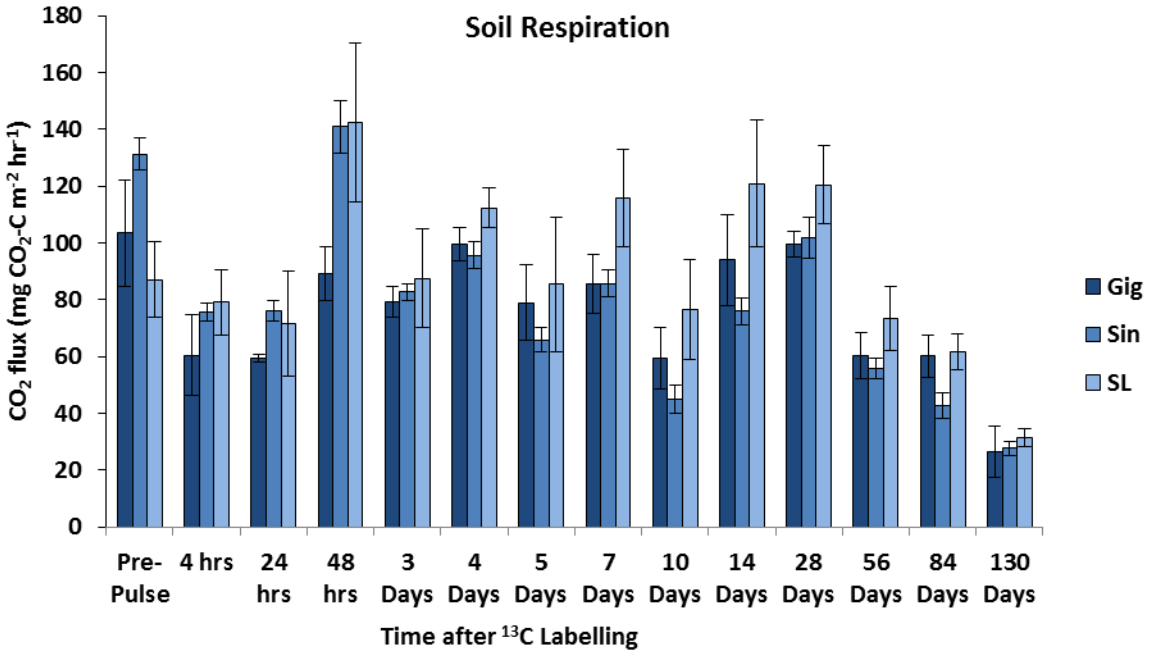

Figure 6.4: Soil respiration: Respiration rates were based on collected chamber gas samples in mg CO_2-C $m^{-2}h^{-1}$ in *Giganteus, Sinensis & Sac/Lut,* pre-pulse to 130 days after the pulse labelling. Results are means and error bars indicate standard errors of the three replicate plots.

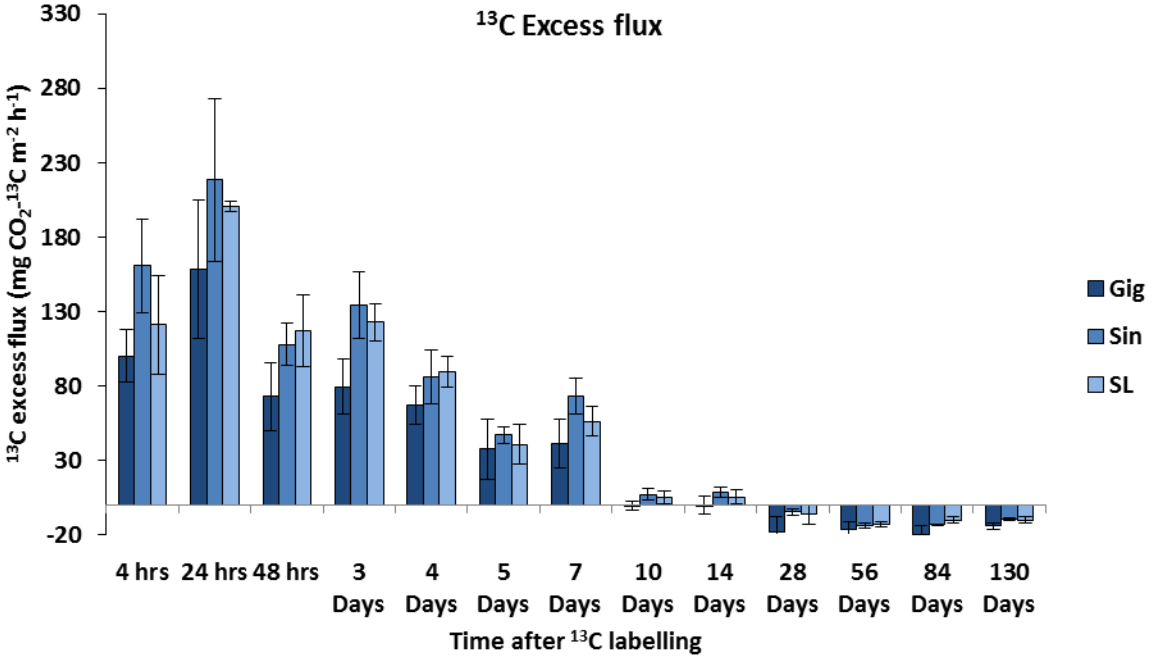

Figure 6.5: ^{13}C soil fluxes: Fluxes were in excess of the natural background flux from *Giganteus*, *Sinensis* and *Sac/Lut.* This data represents the C flux that has arisen from the recently fixed $^{13}CO_2$. Results are means and error bars indicate the standard error of the three replicate plots.

6.3.2 Plant ^{13}C allocation

Enrichment within the top leaves (Figure 6.6 a) of all genotypes peaked between 4 and 24 hours following ^{13}C addition with the rest of the leaves and stems (Figure 6.6 b) peaking slightly later at between ~24 hours to 3 days post-labelling, highlighting the time-lag between fixing of current photosynthate and subsequent transport into other plant structures. Enrichment levels decrease gradually over the sampling time-points in the top leaves with the gradual decrease less evident in the leaves and stems until the final sampling day, 190 days following ^{13}C addition. Significant differences in ^{13}C allocation were observed between sections with the leaves and stems being more enriched than the top leaves section (Table 6.4). It must be noted that some decrease could be due to the dilution of new leaves into the sampling mix. There were no significant differences between *Giganteus* and *Sinensis* enrichment levels in either section of the plant, but there were significant differences with the *Sac/Lut* in 'leaves and stem' (p=<0.05), over time and overall being less enriched than the other two genotypes in above ground biomass (Table 6.5).

Table 6.4: The genotypic and above ground sections (top leaves and leaves & stems) effect on ^{13}C enrichment over 190 days.

	Genotype	Section
^{13}C atom % excess	$F_{2,6} = 5.32$, p = 0.047	$F_{1,151} = 103.74$, p = <0.001

Table 6.5: Significant differences between genotypes over 190 days with regards to above ground vegetation.

Genotype comparison	Estimate	Std error	p-value
Sin – Gig	0.003	0.01	0.96
SL – Gig	-0.04	0.01	0.001
SL – Sin	-0.04	0.01	<0.001

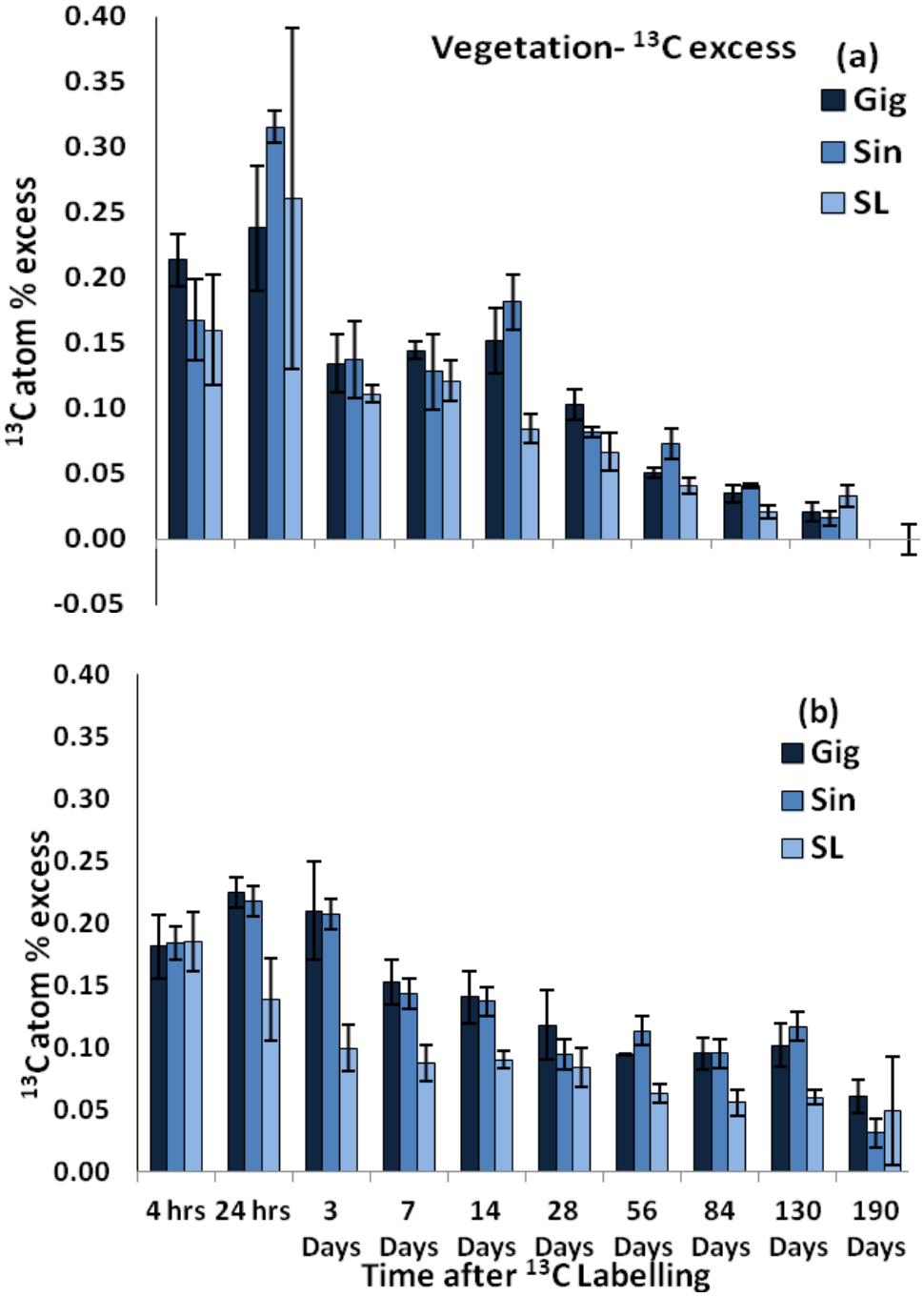

Figure 6.6: ^{13}C atom % vegetation enrichment: Enrichment levels plotted as an excess for (a) top leaves and (b) other leaves and stems structures in *Giganteus*, *Sinensis* and *Sac/lLut* from pre-pulse to 190 days after ^{13}C labelling for the leaves and stem and 130 days in the top leaves due to senescence. Results are means and error bars indicate standard errors for the three replicate plots.

6.3.3 Rhizome & Root ^{13}C allocation

Enrichment in rhizomes peaked at ~24hours after^{13}C labelling, with a gradual decrease up to the final sampling day, and with significant differences evident over time (Figure 6.7a). Although *Sinensis* appeared to maintain the highest level of enrichment overall, due to the variability across the plots, there was no significant difference in rhizome ^{13}C enrichment between the different genotypes (Table 6.6). Excluding three days post ^{13}C labelling, ^{13}C enrichment levels in the roots increased gradually up to 28 days post ^{13}C labelling and then followed a negative trend down to the final sampling day, 190 days after labelling. There were differences evident between genotypes ($F_{2,6}$ = 6.64, p = 0.03), with *Sinensis* being significantly more enriched than Sac/Lut in the roots (Table 6.7).

Table 6.6: Statistics summarising the genotypic effect on ^{13}C enrichment levels in rhizomes and roots over 190 days.

	Rhizomes	Roots
Genotype	$F_{2,6}$ = 3.3, p = 0.108	$F_{2,6}$ = 6.64, p = 0.03

Table 6.7: Statistics summarising the differences between the genotypes on ^{13}C enrichment levels in roots

Genotype comparison	Estimate	Std error	p-value
Sin – Gig	0.012	0.003	0.01
SL – Gig	0.004	0.003	0.40
SL – Sin	-0.007	0.003	0.054

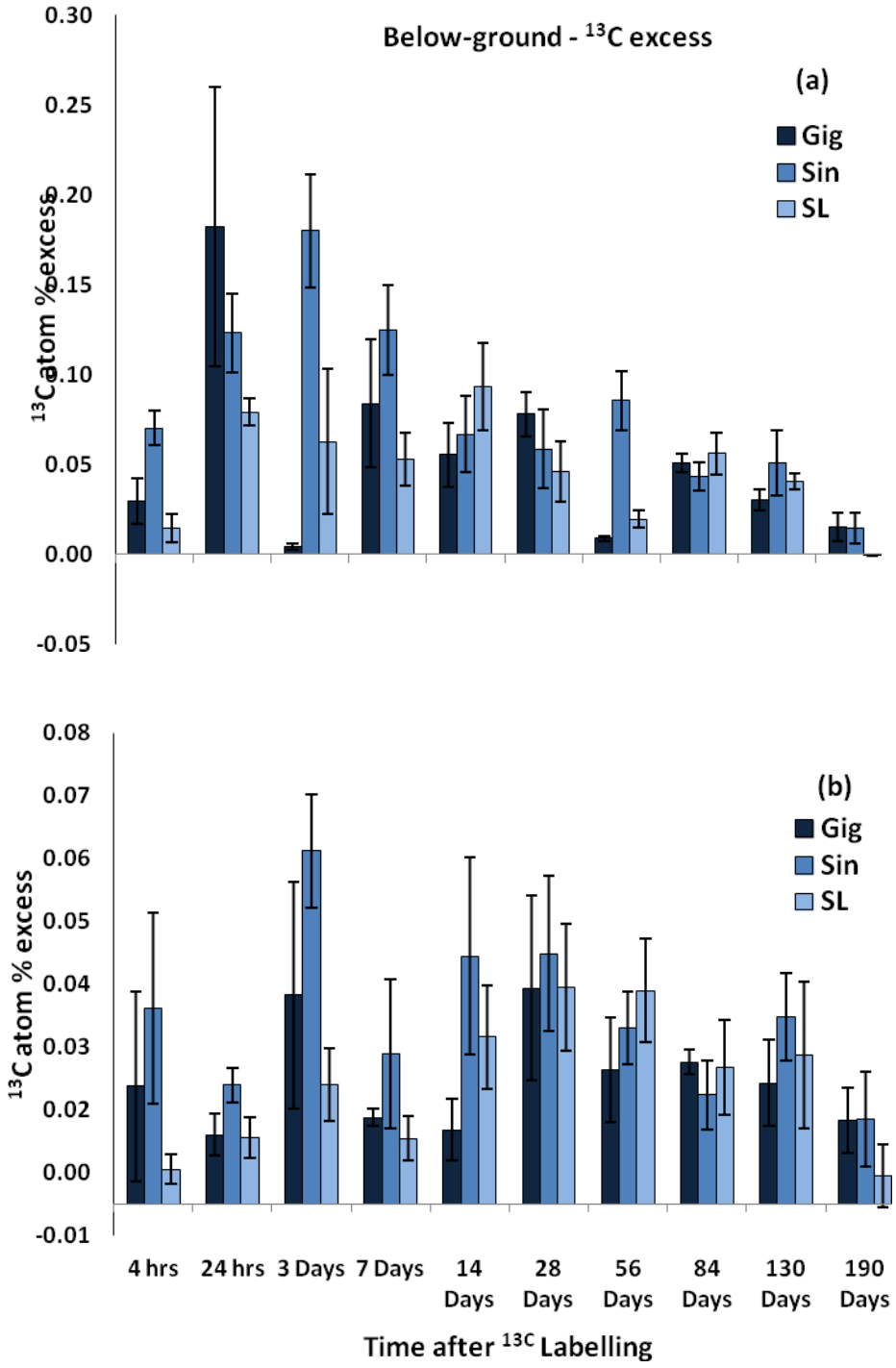

Figure 6.7: ^{13}C atom % enrichment below-ground: Enrichment levels plotted as an excess over natural abundance for (a) Rhizomes and (b) Roots in all *Giganteus*, *Sinensis* and *Sac/lut* from pre-pulse to 190 days after ^{13}C labelling. This data represents newly fixed photosynthate into plant structures and its rate and fate over time. Results are means and error bars indicate standard errors for three replicate plots.

6.3.4 Soil ^{13}C allocation

As expected, the ^{13}C enrichment in the soil pool was low (Figure 6.8). The majority of fixed, pulse-derived ^{13}C was quickly lost through plant and soil respiration with only a very small proportion made available to be added to the soil C pool. There were differences between genotypes, as it appears there was no enrichment whatsoever in the *Giganteus* plots at any depth. The negative values do not mean that there was a reduction in ^{13}C post-pulse, but that there was no or very little enrichment. There was little evidence of enrichment in the *Sinensis* at 10-20 cm and 20-30 cm, while the *Sac/Lut* appeared enriched at all depths through most of the sampling period. Overall, there was no significant difference with depth (Table 6.8), but there were significant differences between genotypes with regard to ^{13}C enrichment (Table 6.9).

Table 6.8: Statistics for the genotypic and depth effect on ^{13}C bulk soil enrichment in *Giganteus, Sinensis & Sac/Lut.*

	Genotype	Depth
^{13}C enrichment	$F_{2,256}$ = 25.77, p = <0.0001	$F_{1,256}$ = 0.61, p = 0.44

Table 6.9: Post-Hoc Tukey test on genotype as a significant factor for bulk soil ^{13}C enrichment under *Giganteus, Sinensis & Sac/Lut.*

Genotype comparison	Estimate	Std error	p-value
Sin – Gig	-0.001	0.0002	<0.001
SL – Gig	0.0005	0.0002	0.065
SL – Sin	0.002	0.0002	<0.001

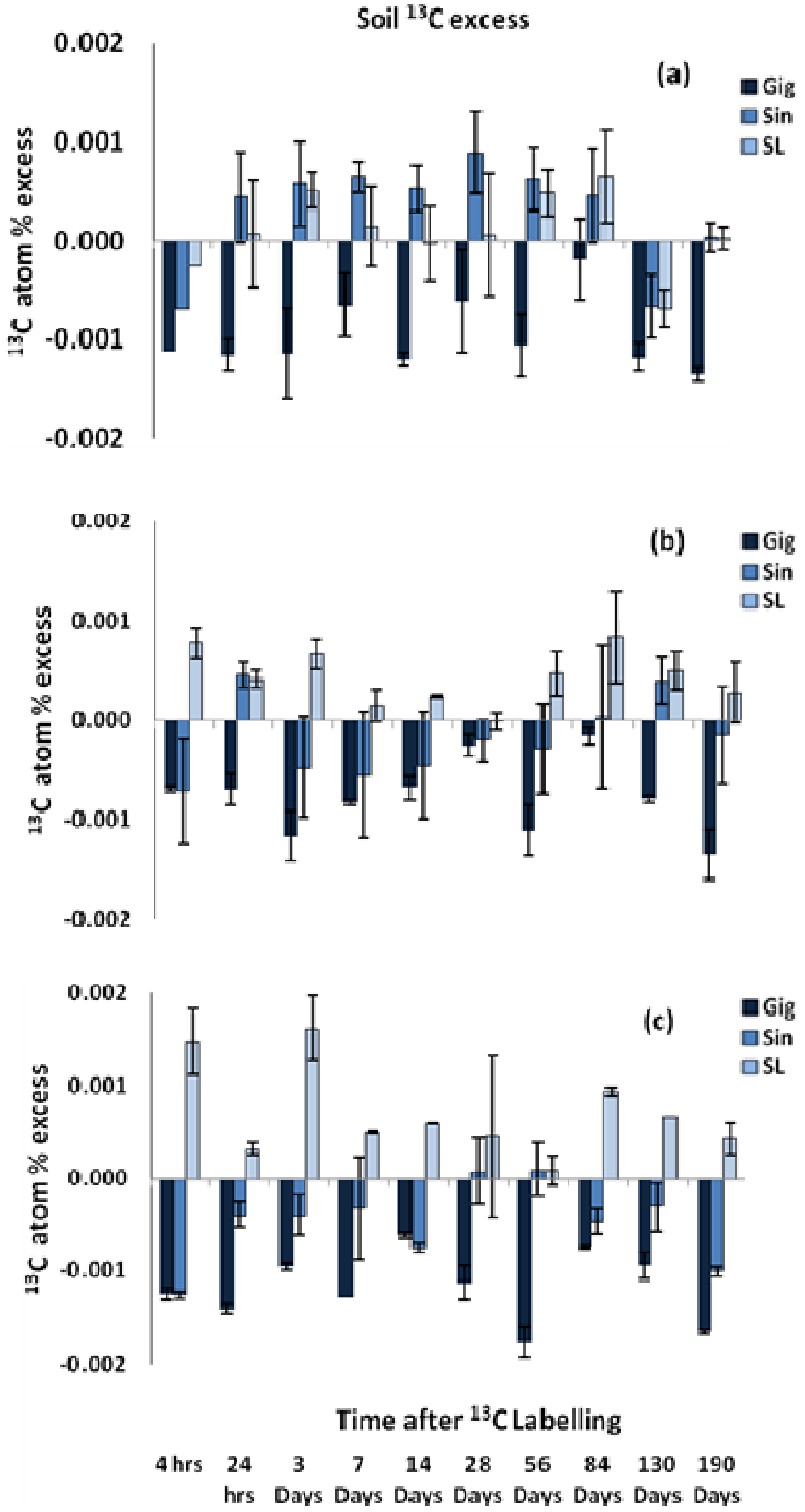

Figure 6.8: ^{13}C atom % enrichment in soil: Enrichment levels plotted as an excess over natural abundance for *Giganteus*, *Sinensis* and *Sac/lut* bulk soil for (a) 0-10 cm, (b) 10-20 cm & (c) 20-30 cm, from pre-pulse to 190 days after ^{13}C labelling. Results are means and standard errors for three replicate plots.

6.3.5 Carbon Pool ^{13}C Allocation

A manipulation of the data was performed in order to consider ^{13}C transport and accumulation within each distinct pool. Top-leaves, bottom-leaves and stems were grouped together to make a total above-ground vegetation pool. Below-ground, roots and rhizomes were keptseperate, while soil depths were bulked giving four carbon pools in total: vegetation, rhizomes, roots and bulk soil. The reason for this grouping was to identify the four individual secions of the terrestrial C cycle and this enabled the comparison of accumulation within each terrestrial C pool.

C pools were significantly different across all genotypes (Table 6.10). All genotype vegetation had significantly higher enrichment over the first 190 days relative to bulk rhizome, roots and soil. Rhizomes had significantly greater enrichment relative to roots and bulk soil, and roots had significantly greater enrichment relative to bulk soil indicating allocation enrichment decreases through the system in all genotypes with greatest C allocation above-ground (Table 6.11).

Table 6.10: Summary table with significant differences for all genotype carbon pools over 190 days. One-way ANOVA with plot/time as random effect to account for repeated measures over time.

Carbon pool	F-value	p-value
Gig	129.61	<.0001
Sin	232.33	<.0001
SL	347.54	<.0001

Table 6.11: Post Hoc Tukey LSD test on each genotype with C Pool as a significant factor.

Genotype	C Pool	Estimate	Std Error	p-value
Gig	Vegetation - Rhizome	0.067	0.004	<0.001
	Rhizome - Roots	0.015	0.004	<0.001
	Roots - Soil	0.02	0.007	<0.001
Sin	Vegetation - Rhizome	0.025	0.007	0.002
	Rhizome - Roots	0.044	0.006	<0.001
	Roots - Soil	0.023	0.002	<0.001
SL	Vegetation - Rhizome	0.037	0.005	<0.001
	Rhizome - Roots	0.034	0.003	<0.001
	Roots - Soil	0.007	0.0003	<0.001

6.3.6 Vegetation C residence times

In order to assess differences in C turnover rates, a simplified C-transfer model with two general pools of carbon was considered: a "fast, labile pool" and a "structural biomass pool" (Subke *et al*., 2012).During the ^{13}C pulse period, a high proportion of CO_2 fixed by the plants was in the form of$^{13}CO_2$ due to the artificially-induced, localised enrichment above natural abundance. Not all C fixed by the plants during the pulse was ^{13}C, as some ^{12}C remained within the tent, along with a constant addition from plant and soil respiration during the enclosure period. Initially, all newly-labelled photosynthate formed part of the "fast labile C pool". This can be respired, form temporary storage in the form of soluble carbohydrates, used for new growth, or allocated to below-ground pools. The remaining ^{13}C becomes incorporated into various structures of the plant and forms a "structural biomass pool". In an attempt to quantify the rate of turnover of the labile C pool, data was fitted to an appropriate mathematical function which yielded the best fit to the data. Previous studies have used both exponential and logarithmic functions to model decay rates following ^{13}C or ^{14}C pulse addition (Leake *et al*., 2006; Subke *et al*., 2006). Data was fitted to exponential, logarithmic and power functions. In this case, a power function was found to best describe the post ^{13}C labelling decline. This is a power-law relationship where one variable varies as a power of another. Power curves were fitted from the time of peak enrichment (24 hours in both sections of the plant) observed to 28 days in top leaves and 130 days in leaves & stems after ^{13}C labelling under the assumption that this time period would best capture the majority of turnover of labile C (Figure 6.9). Power functions follow the general form:

$$F(x) = ax^b$$

Where parameter 'a' is a simple scaling factor which has the effect of moving the values of 'x^b' up or down as a increases or decreases respectively. 'b' represents the exponent which controls the functions rate. Positive exponents represent growth, while negative represent decay. Decay rates can then be calculated by solving the function. The non-zero asymptotes of the fitted curves represent the slower structural biomass pool.

Key assumptions considered when modelling ^{13}C decay are that the measured reduction in ^{13}C enrichment was due solely to losses via leaf respiration, phloem transport to other structures of the plant or incorporation into structural carbon (biomass pool). In reality, some dilution will have occured due to the fixation of unlabelled CO_2 after the ^{13}C pulse.

Estimated half-lives (when y=50% of maximum enrichment) for labile C were calculated both for all genotype top leaves, and for the leavesand stems of the plantin Table 6.12.

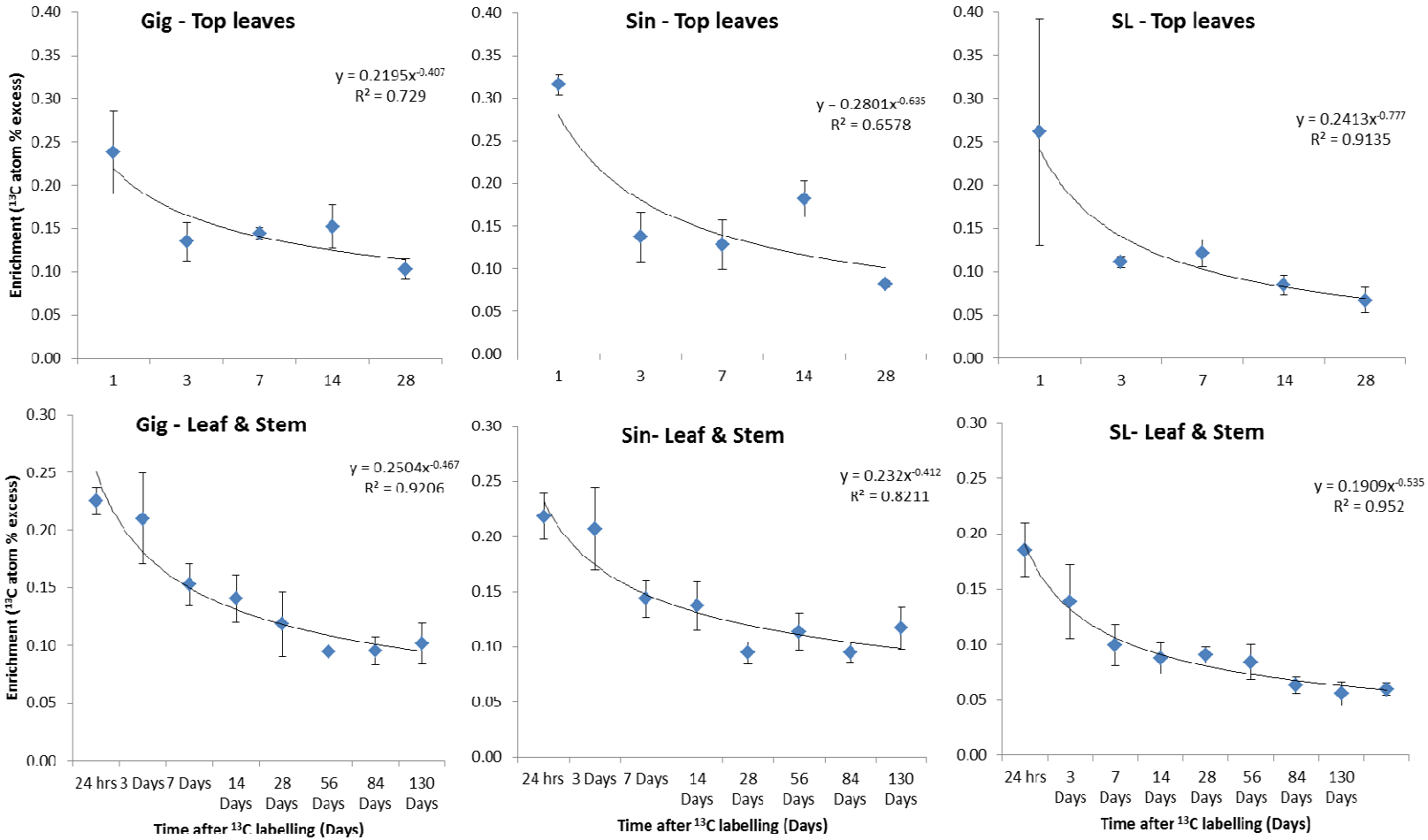

Figure 6.9: ^{13}C atom % excess enrichment in *Giganteus, Sinensis & Sac/Lut* in top leaves up to 28 days and leaves & stems up to 130 days post-^{13}C labelling. Results are means and standard errors of three replicate plots. Lines are fitted power curves for means.

Table 6.12: Summary table of the maximum enrichment, time to reach 50% enrichment and remaining enrichment % at 28 days in top leaves and 130 days in leaves and stems.

Genotype	Section	Max enrich. (Day)	Max enrich. (^{13}C excess)	50% enrich. (Days)	Enrich. at 28 days (%)	Enrich. at 130 days (%)
Gig	Top leaves	1	0.238	4.5	25	
Sin	Top leaves	1	0.316	2.4	9.5	
SL	Top leaves	1	0.261	2.1	7.7	
Gig	Leaves & Stems	1	0.225	5.3		13.3
Sin	Leaves & Stems	1	0.218	6.1		18.3
SL	Leaves & Stems	0.2	0.185	4.1		5.4

6.4 Discussion

This research investigated the C pool allocation and turnover in three different *Miscanthus* genotypes. It was hypothesised that due to the morphological differences in above-ground vegetation that C pool allocation and transfer time between pools would be different. Soil moisture, soil temperature and air temperature all impacted on the soil respiration rates. Decreasing soil moisture was seen to have a significant negative effect which generally coincided with rising air temperatures. (as can be seen in Figure 6.3 and 6.4 in the first 24 hours). The ^{13}C excess flux (Figure 6.5) peaked within 24 hours and then decreased rapidly, with little evidence of enrichment from 10 days post-pulse.

There was a small time-lag between ^{13}C fixed in the top leaves of the plant, and the rest of the plant (Figure 6.6). Enrichment levels decreased,but the gradual decrease was less evident in the other leaves and stems until the final sampling day. The *Sac/Lut* had significantly less enrichment of ^{13}C than the other two genotypes (Figure 6.6), as 50% was lost in the top leaves by day 2, and in the rest of the plant by day 4. On day 130, *Sac/Lut* had only 5.4% enrichment left in its other leaves and stems, while the the *Giganteus* and *Sinensis* had 13.3% and 18.3% left respectively. *Giganteus* took 4.5 days to lose 50% of its enrichment in its top leaves, while the *Sinensis* and *Sac/Lut* had lost 50% on day 2 post-pulse. The differences in the transfer through the *Sac/Lut* plant could have been due to its height which in turn was more affected by the high temperature and low moisture levels pre-pulse (Figure 6.3).

At 24 hours post labelling (excluding day 3 in the case of the roots) it appears that, as the rhizomes lose ^{13}C, the roots appear to gain pulse-derived ^{13}C indicating the peak transfer time from rhizome to root (Figure 6.7); however, the enrichment levels in roots are significantly lower than in the rhizomes in all genotypes.

A probable limitation of this^{13}C pulse-chase approach is that several hours of $^{13}CO_2$ exposure may not always introduce a measurable amount of new C into more

recalcitrant soil pools (Carbone*et al*., 2007; Carbone & Trumbore, 2007; Kuzyakov, 2011). The levels of enrichment in the soil pools for this experiment across all genotypes (Figure 6.8) were near negligible which contrasts with the greater enrichments observed during the previous year's ^{13}C pulse at Lincolnshire (Chapter 5; Figure 5.10). A contributory factor to the lower enrichments observed could be in part due to an extended period of dry weather that preceded the ^{13}C pulse labelling.

Variability observed between all replicate plots may have been caused by the relative amounts of $^{13}CO_2$ initially fixed, which controls to a large degree, the magnitude of allocation of labelled ^{13}C to below-ground root and soil pools (Leake *et al*., 2006). A significant fraction of ^{13}C excess flux measured during the early days post-labelling have been derived from direct diffusion from the soil following exposure to atom % enrichment levels (Johnson*et al*., 2002; Leake et al., 2006). It is important to note that the added ^{13}C was constantly being diluted by unlabelled ecosystem respiration $CO_2$from plants and soil. In addition, the age and position of leaves may have also played a part in the amount of $^{13}CO_2$ ultimately fixed into plant tissues. In August and September the plants were still growing and so the top leaves will have acquired new plant material that was not labelled.

Moving down the plant from above-ground vegetation, to rhizome, to root and finally to the soil, the level of ^{13}C enrichment decreased across all genotypes (Table 6.9). The only significant differences between genotype with regard to C pool allocation was that *Sac/Lut* was less enriched in above-ground biomass than the other two genotype and *Sinensis* was more enriched in the roots. Although there were no consistent significant differences between the genotypes, we can verify the hypothesis that due to above-ground morphological differences between the genotypes, C pool allocation, amounts and transfer time were different.

As stated previously the future of commercial *Miscanthus* will need to incorporate diverse new genotypes in order to adapt to the changing climate. The vulnerability of one genotype if a pest infection ever became an issue would be significant for *Miscanthus*. Therefore as more research, development and commercialisation of different genotypes increases, the need to understand the sustainability of all genotypes is vital. This research indicates that although some differences were evident in C pool allocation and time, above-ground morphological differences did not impact on C stock and soil respiration rates and thus, no genotype was deemed a bigger C-fixer than the others. This outcome is also apparent from concurrent analysis. However, this is a three-year old plot trial, and longer time scale sampling may reveal differences further down the line.

Short ^{13}C pulses are useful for assessing maximum transfer velocity from leaves to other compartments as labile C becomes much more strongly labelled than structural compounds (Liebig *et al*., 2005), while a continuous longer-term (and more costly) ^{13}C labelling approach could prove useful in investigating mean transfer rates through compartments including short term storage pools (Studer *et al*., 2014).

7. OVERALL DISCUSSION

7.1 Filling the knowledge gaps

The primary objective of the measurements described in this report was to inform model development through providing data for the calibration of model parameters and testing of the model's simulations. This was achieved through the development of a network of sites to monitor the effects of land-use change over different transitions and temporal scales. Chamber based methods of measuring CO_2, CH_4 and N_2O were deployed across all network sites, whilst EC equipment was established or maintained at a number of sites throughout the sampling period. Chamber methods refer to the use of IRGAs to measure CO_2 flux and static chambers for measuring CH_4 and N_2O.

With regard to model development, the determination of soil CO_2 flux by chamber methods has some benefits over EC. The chamber method provides a more direct measure of soil respiration, whereas EC data provides TER, which includes respiration from the above-ground biomass. The model developed in Deliverable 4.3 predicts heterotrophic respiration and so CO_2 measurements from either technique had to be partitioned into autotrophic and heterotrophic respiration. Using chamber CO_2 data is preferable as fewer stages of partitioning are required and, in many cases, the partitioning factors applied can be derived from field-based experiments. In addition the chamber technique provided a measure of non-CO_2 GHGs (CH_4 and N_2O), which the EC systems in this project did not have the capabilities to measure. Monitoring of these gases was considered to be of value due to uncertainty regarding the impact of bioenergy cropping systems on fluxes of these potent GHGs, and the requirement for data with which to parameterise the model's CH_4 and N_2O fluxes.

Whilst the original ELUM focus was on soil GHG emissions, it was recognised that measurements of NEE (by EC) would provide additional and valuable results regarding whole CO_2 exchange, taking in to account C uptake by the vegetation and C loss through above- and below-ground respiration. Past and on-going data were leveraged from existing EC systems whilst new systems were commissioned at Lincolnshire (arable) and for both fields at West Sussex. The EC systems provided additional insight, for example, relating increases in soil respiration to changes in C uptake by the crop. In addition, the EC technique provides a continuous measure of C exchange over long temporal scales, allowing the effects of diurnal patterns, environmental factors and growth cycle stage to be examined. Continuous measurements also allow for the calculation of more accurate annual balances whilst chamber measurements provide a greater understanding of point scale GHG variability, as well as capturing information for all three GHGs.

In addition to the monitoring network developed, WP3 also included novel experimental work looking at C allocation within different bioenergy crops. Through this work we aimed to examine whether patterns in net C balances and soil respiration between the different crops could be explained by differences in C usage

and allocation. A ^{13}C pulse labelling technique was utilised, with blocks of SRC willow stools and *Miscanthus x giganteus* plants encased in large tents in which the atmosphere was enriched in $^{13}CO_2$. The ^{13}C was traced into different pools within the plants and soil for 194 days after the one day pulse event. The pulse labelling experiment was a technical challenge in terms of the sheer scale of the field event, and with respect to laboratory developments regarding isotopic analysis of gas and solid samples. This work was repeated during the following year where C uptake and allocation was measured for three *Miscanthus* genotypes.

7.2. Overall GHG balances of land-use change to bioenergy

As previously mentioned the primary objective of WP3 was to deliver soil GHG data to the model developed as part of Deliverable 4.3. These data were used to parameterise and test the model, which can be utilised to predict the soil emissions of CO_2, CH_4 and N_2O annually. Therefore, it is the model output which should be referred to with regard to examining the effects of land-use change on soil GHG emissions. However, from the data collected in WP3 it is possible to make some statements about how transition to bioenergy affects soil GHG emissions. In addition for sites where EC data is available insights can be offered into the overall GHG balances for different land cover types.

Table 7.1: Summary of the annual GHG balances of land cover types at the field sites. Fluxes reported for N_2O and CH_4 are the mean ± 1 std error based upon chamber replication.

Field site	Land cover	NEE (g CO_2 m^{-2})	N_2O (g CO_2 eq m^{-2})	CH_4 (g CO_2 eq m^{-2})
Aberystwyth	*Miscanthus x giganteus* 2012	968	705 ± 120	103 ± 94.8
	Miscanthus x giganteus 2013	-440	233 ± 57.0	10.2 ± 13.2
East Grange	SRF 2012	-3828	7.01 ± 0.46	-0.53 ± 0.12
Lincolnshire	*Miscanthus x giganteus* 2012	-1771	0.02 ± 0.57	0.15 ± 0.26
	SRC willow 2012	-1588	4.92 ± 3.60	-0.54 ± 0.64
	winter wheat	-2439*	31.6 ± 5.32	-0.61 ± 0.32
	spring barley	-906**	41.9 ± 7.42	3.78 ± 3.05
West Sussex	SRC willow 2013	-3234	14.4 ± 2.49	-1.87 ± 0.35
	grass 2013	862	22.8 ± 6.72	1.06 ± 1.49

* 5 April – 8 August 2012
** 25 May – 1 September 2013

Table 7.1 summarises the annual GHG balances for the sites and years for which NEE data was available. The N_2O and CH_4 data derives from the chamber-based measurements at the sites and are based on an approximate calculation of annual flux by up-scaling the monthly measurements of N_2O and CH_4. Up-scaling was achieved by gap filling where data was missing, either with the mean for the month if only a replicate chamber was missing, or with an average of the two months either side of a month where a full dataset was missing. Using this dataset, an "area under the curve" was calculated using the TRAPZ function in R, for each replicate. The values obtained for N_2O and CH_4 are approximate and do not take into account diurnal variation in fluxes, nor potential variation between the measurement points. However, it does allow the potential for GHG emissions from the different sites to be examined and a comparison between NEE and emissions of non-CO_2 GHGs.

7.2.1. Transition from arable to bioenergy

Conversion from arable to bioenergy studied at East Grange and Lincoln showed lower soil microbial CO_2 emissions under both *Miscanthus x giganteus* and SRC willow, when compared to the arable reference. These findings suggest that planting perennial bioenergy crops on arable land reduces microbial driven losses of C. EC data from the Lincolnshire site showed that both arable and bioenergy crops result in a negative NEE, indicating that all crops fixed substantially more CO_2 than was released through respiration processes during the measurement period (Table 7.1). The total NEE shown in Table 7.1 indicate that the winter wheat arable crop has the highest C uptake to release ratio, however the shorter timescale of the measurements covered only the peak growing season. As all of this work was undertaken in commercial fields there were periods where EC equipment could not be installed.

At the Lincolnshire site total soil respiration (including autotrophic root respiration) was significantly lower under *Miscanthus x giganteus* when compared to SRC willow. This reflects the findings of the ^{13}C pulse experiment where higher proportions of the recently assimilated C were retained within the "structural biomass pool" compared to in SRC willow. This means that a greater proportion of fixed carbon through photosynthesis was retained in the biomass. As the crops were at different stages of growth cycle for the ^{13}C pulse care should be taken where comparisons between the crops are being made. Nonetheless these results are the first to examine C dynamics under bioenergy using ^{13}C pulse-chase techniques. The EC data for Lincolnshire demonstrated that TER from the *Miscanthus x giganteus* was lower than for the SRC willow resulting in a more negative NEE. At the Lincolnshire site C stocks beneath the *Miscanthus x giganteus* were greater than beneath both the arable and the willow, potentially reflecting the more efficient C-cycling suggested from the GHG measurements. Across WP2 no net change in soil C stock was observed following arable to bioenergy transitions. However, for Lincolnshire all of these results taken together suggest that *Miscanthus x giganteus* was more efficient than SRC willow at retaining fixed carbon within the plant soil system.

Increased soil respiration in the final year was observed from the Lincolnshire *Miscanthus x giganteus* sub-site, although high within sub-site variability meant that the increase observed was not statistically significant. Higher soil CO_2 emissions would be unsurprising following the harrowing and wood-waste application in Spring 2013 (Section 2.4), as management regimes such as these are known to increase microbial activity and soil CO_2 emissions (Paustian *et al.*, 2000; Dawson & Smith, 2007). Therefore, management matters with more intensive management likely to result in increases in soil GHG emissions.

Nitrous oxide emissions from soils have been a major concern, with uncertainty surrounding the impact of growing certain bioenergy crops (Crutzen *et al.*, 2007); concern for arable to perennial bioenergy crop conversions is most likely unfounded, but robust data are required to underpin all transitions and management practices. Our results showed that the cessation of fertiliser in the perennial crops led to a substantial decrease in N_2O emissions (Section 4.4.2). However, it is possible that perennial crops might be fertilised either during the establishment phase or to maintain productively near the end of the crop life cycle. Limited work based on *Miscanthus* suggests that yield gain (in terms of N_2O savings achieved through biomass utilisation compared to peat and coal) following fertiliser addition is not offset by enhanced N_2O emissions (Roth *et al.*, 2014).

As discussed in Section 4, CH_4 emissions were low from all land-uses at the arable to bioenergy sites, with no difference in flux observed between land-uses. This is unsurprising as CH_4 fluxes from other bioenergy studies have been found to contribute negligible amounts of CH_4 to the overall soil GHG budget.

In conclusion, across the arable to bioenergy transitions lower microbial CO_2 and N_2O production was observed in the bioenergy crops compared to the arable references. Overall NEE was negative for all crops at the Lincolnshire site and data suggest that at this site the *Miscanthus x giganteus* was more efficient than SRC willow at retaining C within the terrestrial system. There are clear benefits with regard to N_2O reduction following transition from arable to bioenergy crops at the sites examined.

7.2.2. Transition from grass to bioenergy

Grasslands can be sinks or sources of CO_2 depending upon factors such as grazing, management and environmental stresses (such as drought and heat) (Gilamanov *et al.,* 2007). As such it is unsurprising that the effect of transition from grass to bioenergy on CO_2 efflux is mixed across the network sites. As previously discussed, the chamber CO_2 data were partitioned into autotrophic (plant and root) and heterotrophic (microbial) respiration. This was to allow for a direct comparison of microbial respiration, rather than an unbalanced comparison of TER from the grassland versus soil respiration in the bioenergy crops.

Heterotrophic CO_2 emissions were found to be higher from the SRC willow land-use at the West Sussex site when compared to the grass control. In contrast, the EC

data for this site shows that the SRC willow acts as a far greater C sink than the grass reference (Section 3 and Table 7.1). This is shown by both higher GPP and substantially lower TER measured over the SRC willow. The contrasting result between the EC and chamber-based techniques reflects the fact the EC measures TER whilst the chamber data has been partitioned to give heterotrophic respiration alone. Comparisons between the EC and chamber data are indicative of higher autotrophic respiration from the grass compared to the willow, with more rapid cycling of recently fixed C in the grass. Similarly in the early stages of transition from grass to SRC bioenergy, Nikièma *et al.* (2012) observed higher CO_2 emissions from a grass reference which they suggest could be attributed to high root respiration. The contribution of autotrophic respiration from grass is highly variable in the literature with root respiration shown to contribute between 10-90% of total ecosystem respiration (Hanson *et al.* 2000). Despite the higher heterotrophic respiration observed from the SRC willow soils, NEE data showed that the SRC willow acted as a substantially greater C sink compared to the grass reference over the measurement period.

The transition from grass to SRF at East Grange showed no overall difference in heterotrophic respiration between the two land-covers. This is likely to be linked to the early establishment phase of the SRF, with the understory of the SRF plantation still similar to the previous grass land-use. It is encouraging to observe that the transition from grass to SRF does not result in substantially higher heterotrophic respiration rates during this phase of the establishment. In addition EC data demonstrates that SRF acts as an efficient C sink, with high levels of GPP compared to TER resulting in the most negative NEE of all the bioenergy crops over which EC was established (Tables 3.2 and 7.1). As with all the bioenergy crops, patterns in GHG emissions may change over the lifetime of the crop and there is value in continuing measurements into the longer term in order to establish the effects of mature plantations on GHG dynamics.

It is extremely valuable to capture soil GHG emissions across the transition phase, as has been achieved at the Aberystwyth site. The transition phase can result in substantial increases in soil emissions of CO_2 and N_2O, with more insight required into the GHG dynamics of this phase for the development of complete LCAs for bioenergy crops (Zona *et al.*, 2013a). EC data from the *Miscanthus x giganteus* field showed that in the establishment year NEE was positive due to high TER relative to GPP (Table 7.1). However, within the first year of establishment, NEE becomes negative, resulting from increases in GPP rather than decreases in TER. This suggests that, at the Aberystwyth site, net C losses following land conversion to *Miscanthus x giganteus* were short-lived and over the lifetime of the crop C uptake will outweigh soil C losses. The chamber data also indicates that transition does not result in significantly higher heterotrophic respiration in the *Miscanthus x giganteus* when compared to the original grass land-use. In the second season following transition, heterotrophic respiration was observed to be significantly higher in the grass compared to the *Miscanthus x giganteus.* Potentially this could result from more efficient C cycling within the *Miscanthus x giganteus* biomass, resulting in

reduced root exudate C-input to microbial communities near the soil surface. However, further experimental work using ^{13}C techniques would be required in order to establish how plant-soil interactions vary between *Miscanthus x giganteus* and grass.

At the second Aberystwyth site a ^{13}C pulse labelling experiment was carried out on three *Miscanthus* genotypes in a replicated block experiment. In similar fashion to the Lincolnshire experiment, ^{13}C enrichment in plant structural biomass pool was observed to decline slowly over the months following the experiment. From above-ground vegetation to rhizome to root and into the soil, the level of ^{13}C enrichment decreased in all genotypes as photosynthate transferred from pool to pool. For this experiment, little incorporation of ^{13}C was observed in the soil pool which contrasts with that observed at Lincolnshire. It is possible that the lower enrichments observed could be in part due to an extended period of dry weather that preceded the ^{13}C pulse labelling. Overall, whilst some differences were observed for ^{13}C in plant pools, no significant differences were observed for ^{13}C in soil or soil respiration. This reflects the results from the two years of soil respiration measurements. As these crops were at a young age (3 years), it is possible that as they develop, genotypic variations might impact on the C cycle. This study is, however, the first to assess the impacts of genotypic variation on C cycling.

Concerns regarding N_2O emissions following the establishment of bioenergy crops on grass are generally associated with the transition phase (Nikièma *et al.*, 2012; Zona *et al.*, 2013a,b; Palmer *et al.*, 2013). Nikièma *et al.* (2012) reported increases in N availability and associated N_2O emissions following the establishment of SRC crops on grassland, however they observed that these fluxes were curtailed within a year of establishment. N_2O emissions from the *Miscanthus x giganteus* plots were significantly higher than for the grass reference at the Aberystwyth site. However due to observations of high N_2O emissions from the site prior to conversion it is not possible to attribute this difference to the conversion process. Management processes associated with the conversion from grasslands to bioenergy (such as harrowing and addition of herbicides) are known to increase soil N availability resulting in N_2O emissions (Palmer *et al.*, 2013). At the East Grange and West Sussex sites N_2O fluxes were generally low with no significant differences between the grass reference and the bioenergy crop. Based upon observations at these sites (and from the other network sites) we would conclude that after the establishment period, bioenergy land-use does not result in higher N_2O emissions compared to the previous land-use. Similarly Roth *et al.* (2013) found that in the medium to longer term *Miscanthus x giganteus* establishment had neutral effects with regard to N_2O emissions.

Echoing similar studies (Drewer *et al.*, 2012; Nikièma *et al.*, 2012), CH_4 fluxes were found to be only a minor component of the soil GHG emissions in all land-uses with no association between CH_4 fluxes and bioenergy land-use.

In conclusion for the sites examined, transition from grass to bioenergy does not appear to result in sustained increases in soil microbial respiration. The exception to this is the SRC willow site, but the higher heterotrophic respiration did not equate to overall C losses from the system as it was offset by higher GPP. N_2O and CH_4 emissions were not of significant concern with the established sites (East Grange and West Sussex). At the Aberystwyth transition experiment higher N_2O emissions from the bioenergy crop compared to the grass may be partially attributed to the conversion process but could also reflect the high N_2O emissions observed from the site prior to conversion.

8. CONCLUSIONS AND KEY FINDINGS

The network of measurement field sites is unique because of the diversity of bioenergy crops and other land covers that it includes. The network of sites is also unique in focusing on commercial-scale plots as opposed to field plot trials. Key outcomes and conclusions from this work include:

- Data provided to WP4 has been successfully used for model development and testing.

- Across the arable to bioenergy transitions, lower microbial CO_2 and N_2O production was observed in the bioenergy crops compared to the arable references. Overall NEE was negative for all crops at the Lincolnshire site and data suggest that at this site the *Miscanthus x giganteus* was more efficient than SRC willow at retaining C within the terrestrial system. There are clear benefits with regard to N_2O reduction following transition from arable to bioenergy at the sites examined.

- For the transition from grass to bioenergy there was no sustained increase in soil microbial respiration, with the exception of the SRC willow site. However, at the willow sites the higher heterotrophic respiration did not equate to overall C losses from the system as it was offset by higher GPP in the SRC willow. N_2O and CH_4 emissions were not of significant concern with the established sites (East Grange and West Sussex).

- Across all bioenergy land-uses, fluxes of CH_4 and N_2O were shown to be close to negligible. At the Aberystwyth transition experiment higher N_2O emissions from the bioenergy crop compared to the grass may partially be attributed to the conversion process, but could also reflect the high N_2O emissions observed from the site prior to conversion.

- The pulse experiment at the Lincolnshire site demonstrated that in the case of *Miscanthus x giganteus*, a greater proportion of recently fixed ^{13}C appears to be retained within the “structural Biomass pool” relative to SRC willow. This can partially be attributed to differences in growth phase between the two but may also indicate greater carbon use efficiency of *Miscanthus x giganteus*. At the Aberystwyth site, whilst differences between Miscanthus genotypes C pool allocation were observed, no impact was found for ^{13}C allocation to the soil or for soil ^{13}C respiration rates.

9. RECOMMENDATIONS AND FUTURE RESEARCH REQUIREMENTS

- Continuation of GHG monitoring at the network sites to develop long-term datasets capturing a significant proportion of the life-time of different bioenergy crops and transitions. Long-term measurements provide more information on issues such as: the timing of achieving equilibrium on soil C stocks, changes due to different meteorological conditions and the impact of changes in management practices.

- There is much uncertainty regarding CO_2 source partitioning and research in general is required on this topic in order to make more accurate comparisons of heterotrophic respiration between different crops.

- There is a need for high-resolution measurements of GHG fluxes in order to capture temporal and spatial variation in emissions (Deliverable 3.4), particularly in order to determine the annual N_2O budgets for arable fields. This ELUM work has already led to further opportunities, at the Lincolnshire site in 2014, to carry out high temporal resolution chamber and EC N_2O measurements as part of the NERC GREENHOUSE project.

- Repeat ^{13}C pulse-labelling of crops at different stages of the growth-cycle would benefit our understanding of the plant-soil carbon dynamic for SRC willow and *Miscanthus x giganteus* at Lincolnshire. Follow-on work to resample the Aberystwyth genotype pulse experiment in 2014 would increase understanding of carbon (^{13}C) remobilisation from one growing season to another.

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APPENDIX 1 – SUPPLEMENTARY INFORMATION FOR SECTION 5

A1.1 Soil Respiration $\delta^{13}C$

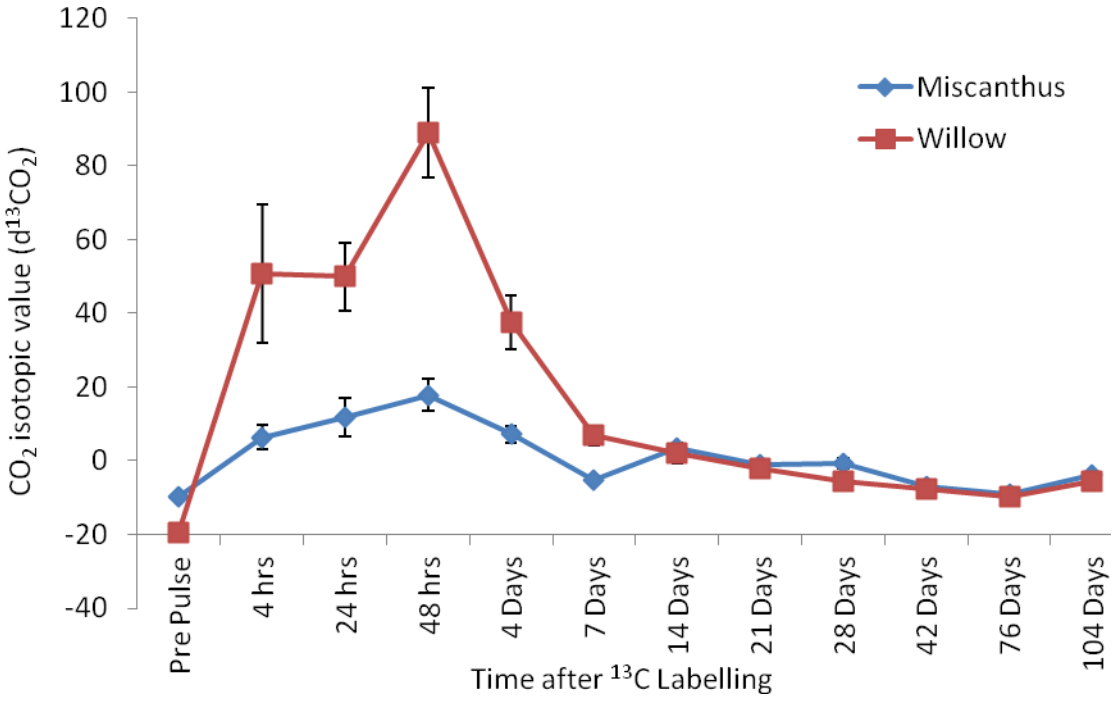


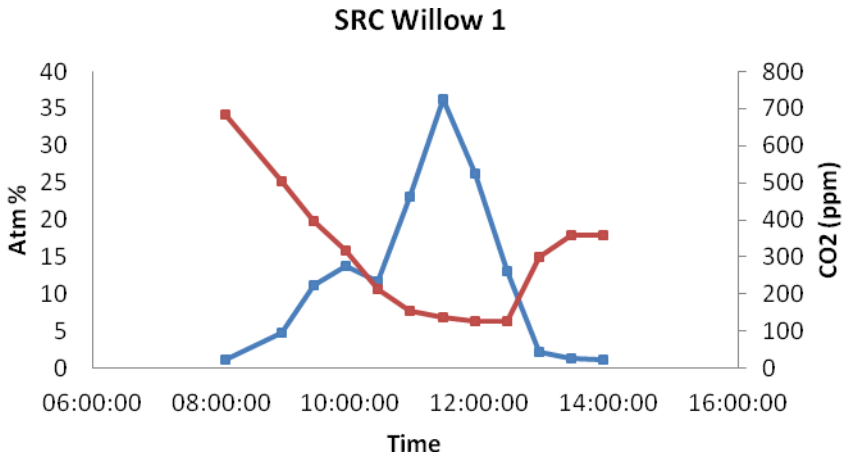
Figure A1.1: $\delta^{13}C$ of Soil Respiration under *Miscanthus* and SRC Willow. Results are means and error bars represent ±1SE for four replicate plots.

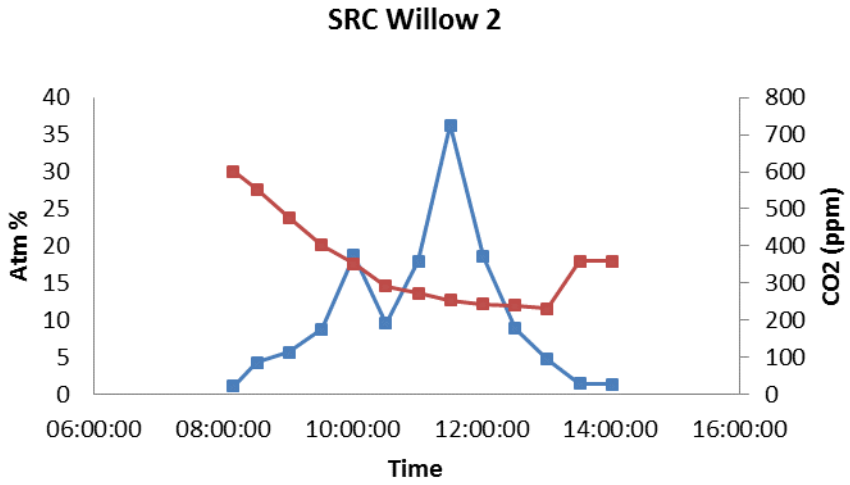
A1.2 Sampling Dates

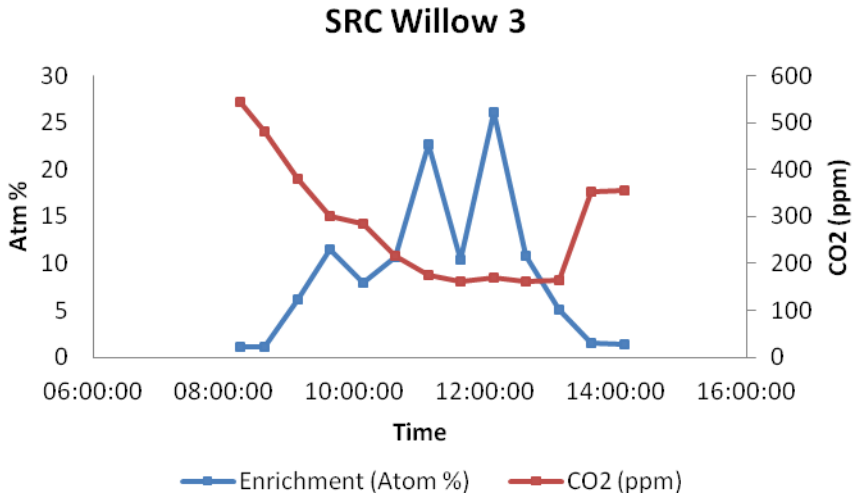
Table A1.1. – Summary table of time-points and sampling dates

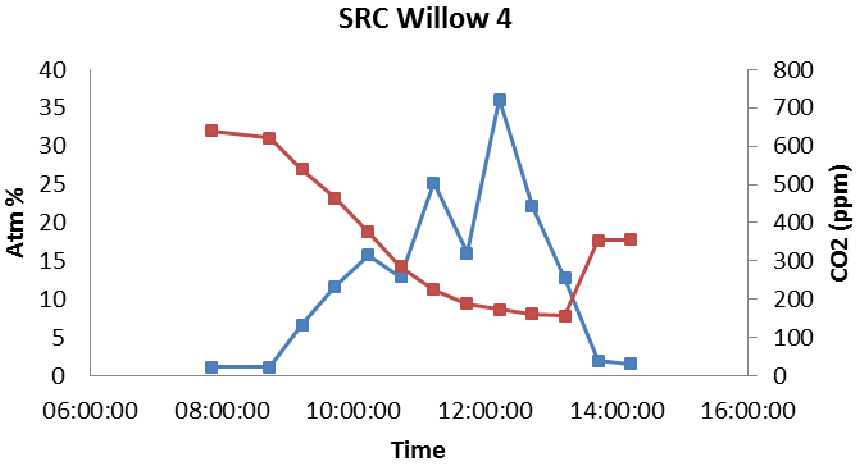
Sampling Point	Date
Pre-pulse	20-Aug-12
4 hrs	23-Aug-12
24 hrs	24-Aug-12
48 hrs	25-Aug-12
4 Days	27-Aug-12
7 Days	30-Aug-12
14 Days	06-Sep-12
21 Days	13-Sep-12
28 Days	20-Sep-12
42 Days	04-Oct-12
76 Days	07-Nov-12
104 Days	05-Dec-12
194 Days	05-Mar-13

A1.3. Ambient Tent air ^{13}C enrichments and CO_2 concentrations during pulse labelling









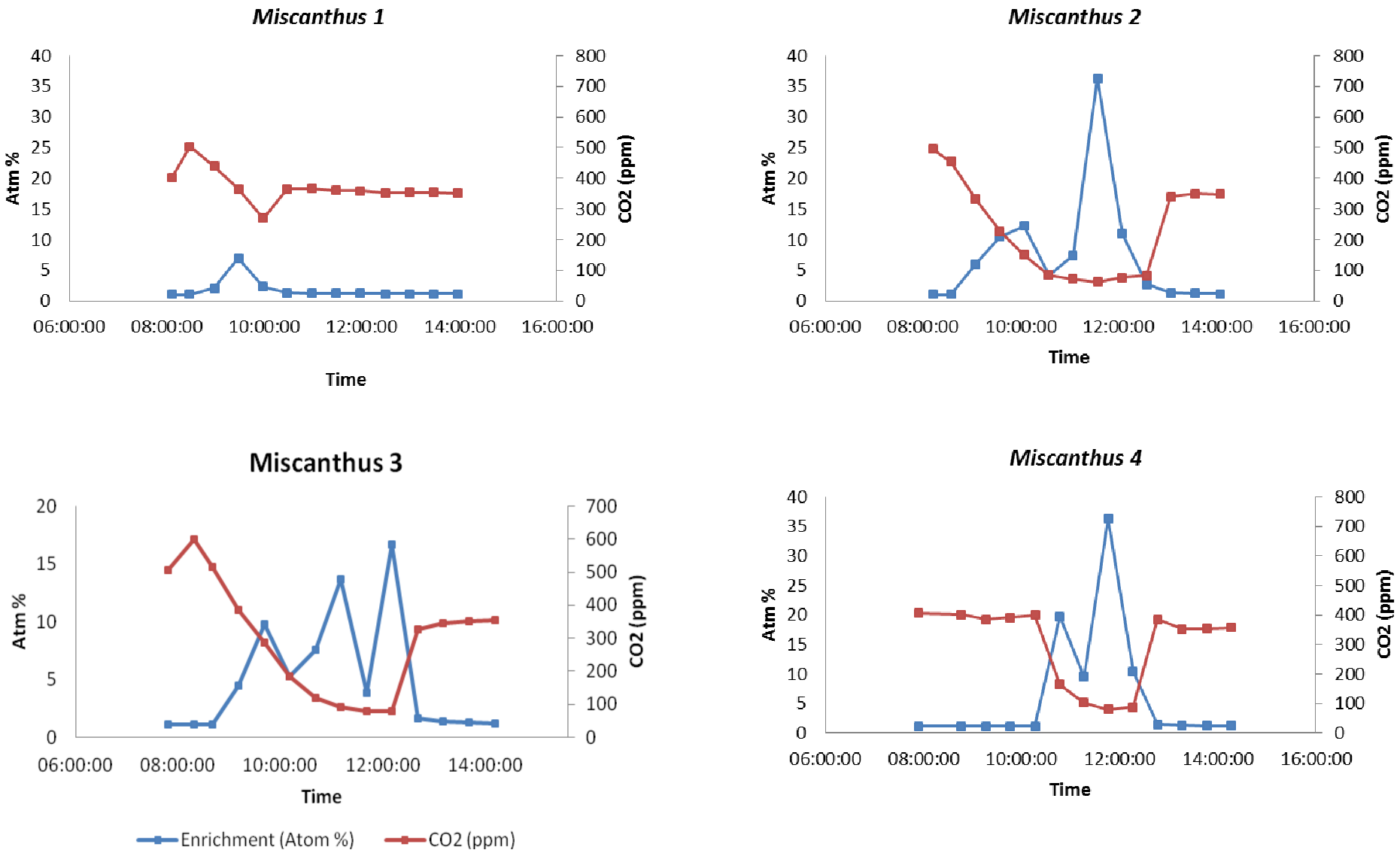
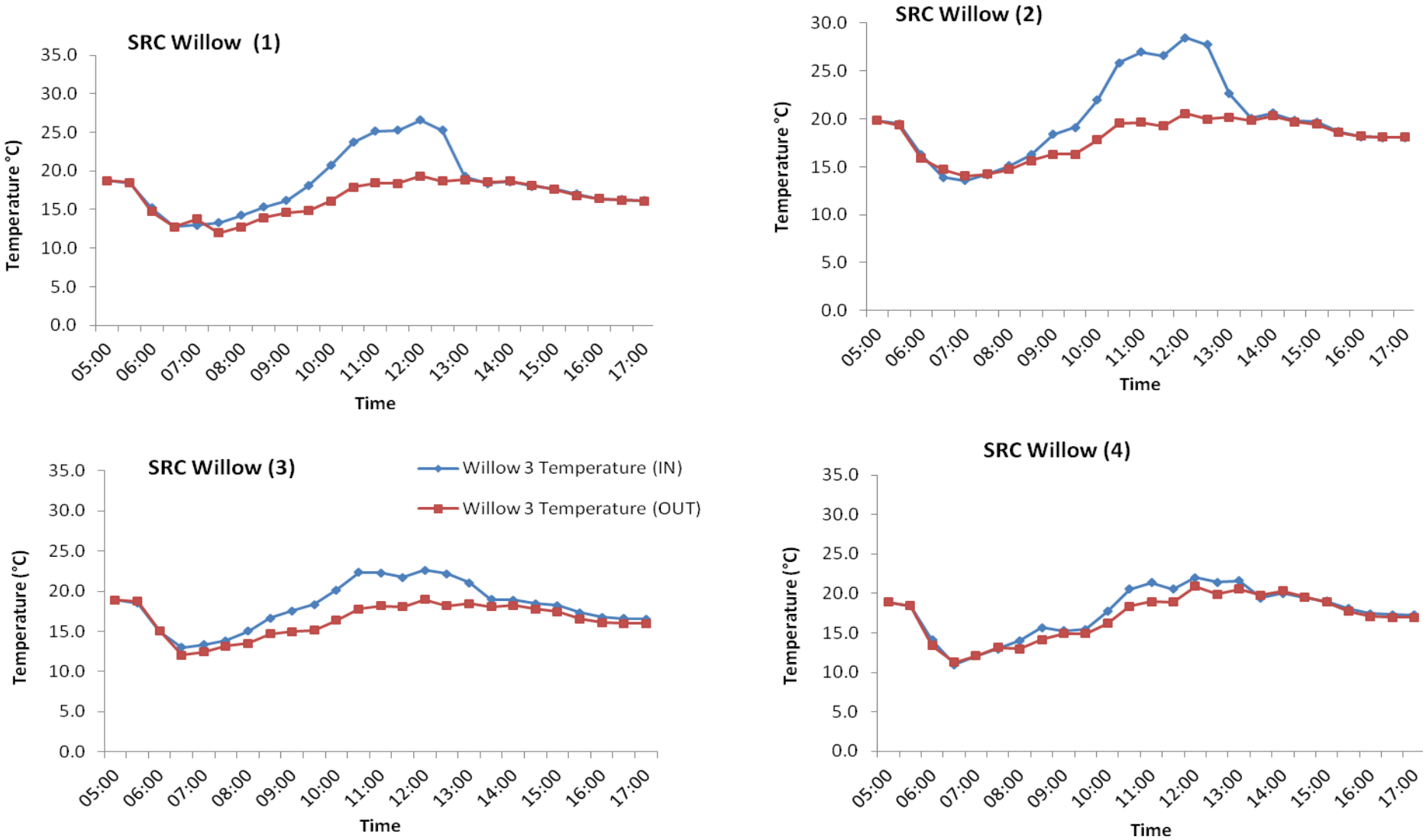


Figure A1.2: Ambient tent air enrichments (Atom %) and CO_2 concentrations (ppm) for all tents, 4 SRC Willow, 4 *Miscanthus x giganteus* during the pulse labelling. Blue lines represent Atom % enrichment. Red lines represent CO_2 concentration.

Figure A1.4 Ambient and tent air temperatures during pulse labelling



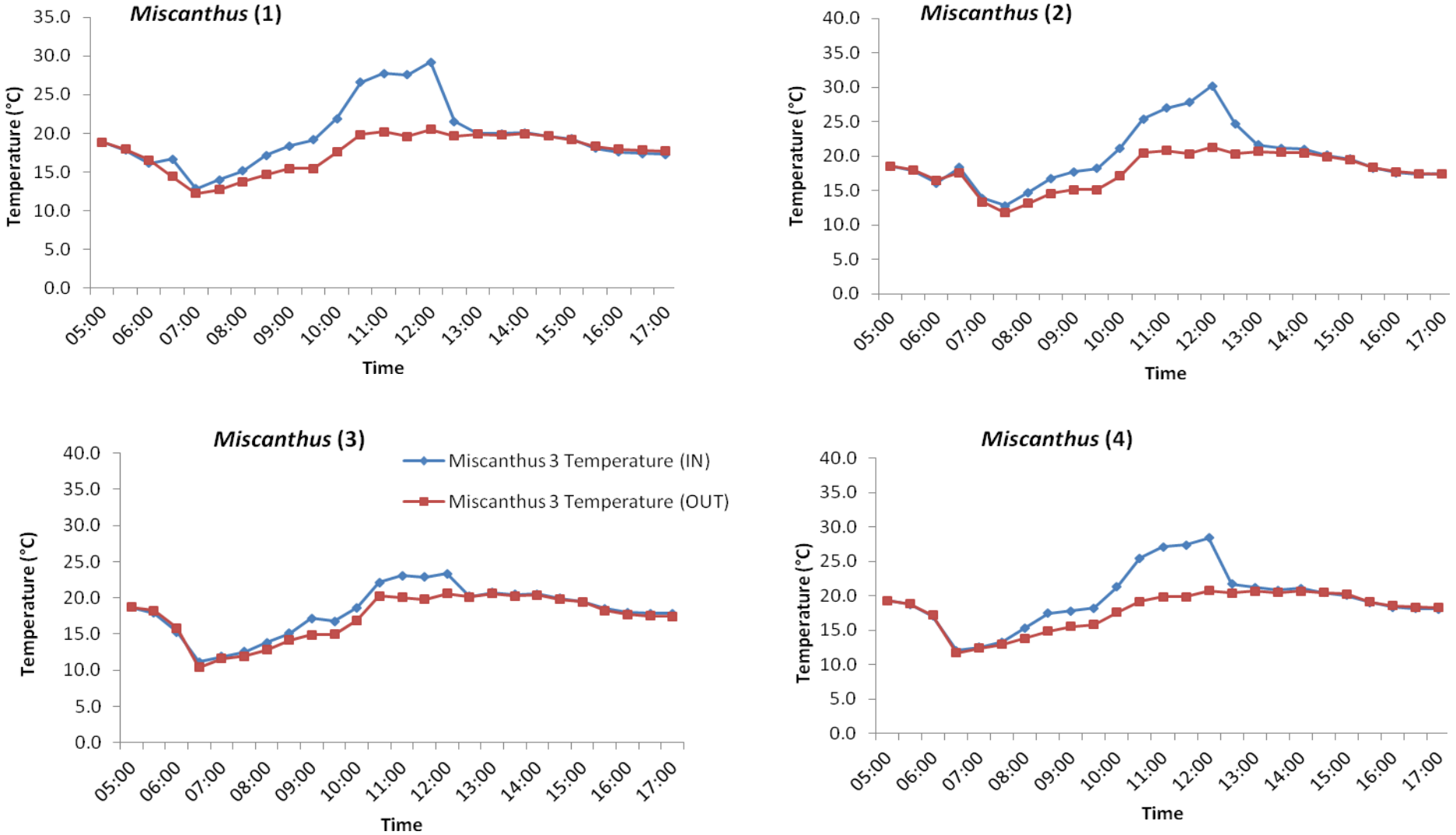


Figure A1.3: Ambient and tent air temperatures for all tents, 4 SRC Willow, 4 *Miscanthus* during the pulse labelling. Blue lines represent temperatures inside the tent, Red lines represent temperatures outside the tent.

A1.5 – Sample R Code

```
*setwd*
  library(nlme)
require(car)
library (multcomp)
require (ggplot2)
library(plyr)
require (grid)
library(scales)
require(RColorBrewer)
CO2.Flux = read.table("CO2 Efflux R.txt", header=T, sep="\t")
str(CO2.Flux)
summary(CO2.Flux)
CO2.Flux$Timepoint=factor(CO2.Flux$Timepoint)
CO2.Flux$tent=factor(CO2.Flux$tent)
CO2.Flux$chamber=factor(CO2.Flux$chamber)
lme_CO2.Flux=lme((CO2.Efflux)~Moisture*Soil.Temp,
  random=~1|tent/chamber, data=CO2.Flux,
  na.action=na.exclude,control=lmeControl(msMaxIter = 200),
  weights=varIdent(form= ~1|Timepoint))
E2 = resid(lme_CO2.Flux, type = "normalized")
F2 = fitted(lme_CO2.Flux)
op = par(mfrow = c(2, 2), mar = c(4, 4, 3, 2))
qqnorm((E2),main="Q-Q")
plot(x = F2, y = E2, xlab = "Fitted values", ylab = "residuals")
plot(E2 ~ Moisture, data = lme_CO2.Flux$data,
       main = "Moisture", ylab = "Residuals")
plot(E2 ~ Soil.Temp, data = lme_CO2.Flux$data,
    main = "Soil Temp", ylab = "Residuals")
shapiro.test(E2)
anova(lme_CO2.Flux)
summary (lme_CO2.Flux)
```

APPENDIX 2 – SUPPLEMENTARY INFORMATION FOR SECTION 6

Table A2.1.– Summary table of time-points and sampling dates

Sampling Point	Date
Pre-pulse	18-Jul-13
4 hrs	26-Jul-13
24 hrs	27-Jul-13
48 hrs	28-Jul-13
3 Days	29-Jul-13
4 Days	30-Jul-13
5 Days	31-Jul-13
7 Days	02-Aug-13
10 Days	05-Aug-13
14 Days	09-Aug-13
28 Days	23-Aug-13
56 Days	13-Sep-13
84 Days	18-Oct-13
130 Days	03-Dec-13
190 Days	03-Feb-14

APPENDIX 3 – GLOSSARY

AGB	Above-Ground Biomass
ASCII	American Standard Code for Information Interchange
BD	Bulk Density
BIO	Biomass
C	Carbon
CEH	Centre for Ecology & Hydrology
CH_4	Methane
CN	Carbon Nitrogen
CO_2	Carbon Dioxide
CO_2-C	Carbon Dioxide as Carbon
csv	Comma Separated Value
DOC	Dissolved Organic Carbon
DPM	Decomposable Plant Material
E	Relative Error
EC	Eddy Covariance
ECA&D	European Climate Assessment & Dataset
ECOSSE	Model to Estimate Carbon in Organic Soils – Sequestration & Emissions
ELS	Entry Level Stewardship
ELUM	Ecosystem Land Use Modelling
FR	Forrest Research
FRS	Functional Requirements Specification
GC	Gas Chromatograph
GHG	GreenHouse Gas
GIS	Graphic Information System
GOR	Government Office Regions
GPP	Gross Primary Productivity
GUI	Graphical User Interface
GWP	Global Warming Potential
ha	hectare
HUM	Humus
HWSD	Harmonized World Soil Database
IOM	Inert Organic Matter
IRGA	Infra-Red Gas Analyser (chamber measurements)
K	Potassium
LCA	Life Cycle Analysis
LOFIT	Lack Of Fit
LRF	Long Rotation Forestry
LUC	Land-Use Change
M	Mean Difference
N	Nitrogen
N_2O	Nitrous Oxide
NEE	Net Ecosystem Exchange
NERC	Natural Environment Research Council

NH_4^+	Ammonium
NO_3^-	Nitrate
NPP	Net Primary Production
NRL	no root/litter plots
odt	Oven Dry Tonne
OSR	Oil Seed Rape
P	Phosphorus
PET	Potential EvapoTranspiration
PM	Payment Milestone
PTF	PedoTransfer Functions
QC	Quality Control
R	Correlation coefficient
Ra	Autotrophic Respiration
Rh	Heterotrophic Respiration
RMS	Root Mean Squared Deviation
RPM	Resistant Plant Material
sd	Standard Deviation
SGR	Stage Gate Review
SO_3	Sulphur Trioxide
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SRC	Short Rotation Coppice
SRF	Short Rotation Forestry
std err	Standard Error
SUG	Sugar Beet
TER	Total Ecosystem Respiration
UK	United Kingdom
UKCP09	UK Spatially Coherent Projections
UKERC	UK Energy Research Centre
WHE	Wheat
WP	Work Package