Functional differences in the microbial processing of recent assimilates under two contrasting perennial bioenergy plantations

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ABSTRACT

Land use change driven alteration of microbial communities can have implications on belowground C cycling and storage, although our understanding of the interactions between plant C inputs and soil microbes is limited. Using phospholipid fatty acids (PLFA's) we profiled the microbial communities under two contrasting UK perennial bioenergy crops, Short Rotation Coppice (SRC) willow and *Miscanthus Giganteus* (miscanthus), and used ^{13}C – pulse labelling to investigate how recent carbon (C) assimilates were transferred through plant tissues to soil microbes. Total PLFA's and fungal to bacterial (F:B) ratios were higher under SRC willow (Total PLFA = 47.70 ± 1.66 SE μg PLFA g^{-1} dry weight soil, F:B = 0.27 ± 0.01 SE) relative to miscanthus (Total PLFA = 30.89 ± 0.73 SE μg PLFA g^{-1} dry weight soil, F:B = 0.17 ± 0.00 SE). Functional differences in microbial communities were highlighted by contrasting processing of labelled C. SRC willow allocated 44% of total ^{13}C detected into fungal PLFA relative to 9% under miscanthus and 380% more ^{13}C was returned to the atmosphere in soil respiration from SRC willow soil compared to miscanthus. Our findings elucidate the roles that bacteria and fungi play in the turnover of recent plant derived C under these two perennial bioenergy crops, and provide important evidence on the impacts of land use change to bioenergy on microbial community composition.

1. Introduction

More carbon (C) is retained within soil than in living vegetation and the atmosphere combined (Jobbagy and Jackson, 2000). At steady state, ecosystem C inputs derived from the photosynthetic assimilation of carbon dioxide (CO_2) are roughly balanced by C losses. C is lost through autotrophic and heterotrophic respiration and to a lesser extent, erosion and leaching of dissolved and particulate C compounds (Davidson and Janssens, 2006). Ecosystem perturbations such as land use change, can alter the magnitude and direction of soil - atmosphere C exchange driving ecosystems towards a new steady state (Anderson-Teixeira et al., 2009; Guo and Gifford, 2002). Alteration of vegetation and associated plant inputs,

and a shift in soil abiotic conditions can drive changes in soil microbial community composition, modifying the nature of the interactions between above and below ground ecosystem components (Bronick and Lal, 2005; De Deyn et al., 2008).

Plant taxa vary widely in the rates at which they assimilate C during photosynthesis; partition C to above and below - ground structures, lose C through senescence, root exudation and respiration and drive changes in soil abiotic conditions (Cornwell et al., 2008; De Deyn et al., 2008; Dorrepaal, 2007). In addition to differences in metabolic pathways between taxa, the efficiency with which plants assimilate C is also dependent upon both environmental conditions and the phenological stage (Desalme et al., 2017; Gowik and Westhoff, 2011; Raines, 2011).

Previous isotopic tracer studies have shown a strong coupling between recently fixed plant photosynthates and belowground C allocation to roots, soil microbes and respiratory fluxes (Bahn et al., 2013; Barthel et al., 2014; Carbone et al., 2007; Epron et al., 2011; Hogberg et al., 2008; Jin and Evans, 2010; Sommer et al., 2016;

Street et al., 2013; Subke et al., 2012; Tavi et al., 2013; Weng et al., 2017). The fate of this C is ultimately controlled by the composition and activity of soil biota, however our understanding of the below ground C flux and its partitioning amongst roots, mycorrhizae and free living soil microbes is still limited (De Deyn et al., 2008; Stuart Chapin et al., 2009). Different microbial groups have however been associated with specific functions; Gram negative bacteria are commonly associated with the rhizosphere and the decomposition of root exudates (Denef et al., 2009; Koranda et al., 2014) whilst Gram positive bacteria are predominant in bulk soil and can decompose older, more complex soil organic matter (SOM) (Bahn et al., 2013). Saprotrophic fungi have wide enzymatic capabilities and predominate in the decomposition of complex organic compounds in plant residues (Baldrian and Valášková, 2008) whilst arbuscular (AMF) and ectomycorrhizal fungi (EMF) are obligate symbionts and exchange C for nutrients with their hosts (Denef et al., 2007; Drigo et al., 2010; Treseder and Allen, 2000). Importantly, soil biota are sensitive to changes in plant diversity with plant community manipulations and changes in land use having been shown to alter microbial communities, rates of soil microbial C turnover, storage and ecosystem functions (Mellado-Vazquez et al., 2016; Steinauer et al., 2015).

In the case of biomass production for bioenergy and biofuels, such changes in microbial C turnover are of particular interest as potential benefits for soil C have been used as a rationale for switching from annual to perennial systems for the production of biomass (Adler et al., 2007). Perennial cropping systems offer the advantage of reduced physical disturbance after crop establishment, with a lack of annual tillage promoting stable soil biotic communities and potentially, minimizing soil carbon loss (Tiemann and Grandy, 2015). However, perennial bioenergy systems represent a significant change in plant taxa, land use and management with the impacts upon soil microbial community structure and function still relatively poorly studied (Cattaneo et al., 2014; Hargreaves and Hofmockel, 2014; Mao et al., 2013). Two of the leading temperate, perennial biomass crops are the hybrid *Miscanthus Giganteus* (miscanthus), a rhizomatous, woody perennial C4 grass native to Asia (Rowe et al., 2009) and short rotation coppice (SRC) willow, a C3 tree native to the United Kingdom which is grown in high density plantations (Hilton, 2002). These have been proposed to be planted across Europe as dedicated bioenergy crops, primarily as a low-carbon substitute for fossil fuels in thermal power generation (Don et al., 2012; Kahle et al., 2001). There remains considerable uncertainty regarding the impact on soil C, of land use change to these bioenergy crops. Walter et al. (2015) reported rates of change of $-1.3-1.4$ Mg C ha^{-1} yr^{-1} across 21 SRC willow plantations in central Europe. For miscanthus transitions, Poeplau and Don (2014) reported a range of $-0.17-1.54$ Mg C ha^{-1} yr^{-1} whilst Rowe et al. (2016) reported rates of change of 1.54 ± 0.70 Mg C ha^{-1} yr and -0.93 ± 0.74 Mg C ha^{-1} yr across arable to SRC willow and miscanthus transitions respectively. Much of this variability can be accounted for by the relatively young age of crops, differences in previous land uses and abiotic factors, however an understanding of how biotic processes may control C turnover under these crops is currently lacking. For example, whilst it is suggested that globally, ecosystems dominated by ericoid and EMF can hold more soil C than those dominated by AMF (Averill et al., 2014) no research has considered whether EMF association in SRC Willow processes and sequesters assimilated carbon differently to AMF associating miscanthus.

Utilizing established plantations of miscanthus and SRC willow, which exhibit contrasting photosynthetic pathways, growth rates and environmental tolerances (Larsen et al., 2016; Quinn et al., 2012; Bellarby et al., 2010), we applied ^{13}C - pulse labelling to investigate C assimilation and the transfer, distribution and persistence of plant derived C within microbial communities. ^{13}C - pulse labelling has previously been applied to agricultural systems (Tavi et al., 2013), grasslands (De Deyn et al., 2011; Leake et al., 2006; Ostle et al., 2000), peatlands (Biasi et al., 2012; Fenner et al., 2004; Ward et al., 2009) and forest ecosystems (Epron et al., 2011; Hogberg et al., 2008; Subke et al., 2009). Few tracer studies of this kind have been performed on energy crops (Chaudhary et al., 2012; Horwath et al., 1994; Mikan et al., 2000; Tavi et al., 2013) and none, to our knowledge on miscanthus and SRC willow.

We predicted that miscanthus may have greater carbon use efficiency (CUE) than SRC willow due to its C4 photosynthetic pathway, leading to a reduction in below ground C transport, rhizodeposition and C transfer to microbial communities as measured by ^{13}C $-$ PLFA and soil ^{13}CO$_2$ flux. We also hypothesised that C would be transferred below - ground more rapidly under miscanthus relative to SRC willow as previous studies have shown slower transfer rates in trees (Hogberg et al., 2008) relative to tall perennial grasses (Tavi et al., 2013). Finally, we predicted that below - ground C allocation would be greater under SRC Willow due to its association with ectomycorrhizal fungi (EMF), which have been shown previously, to be strong sinks for photosynthates in forest systems (Hogberg et al., 2008).

2. Materials and methods

2.1. Research site

The research sites were two adjacent commercial bioenergy plantations of miscanthus (11.56 Ha) and SRC willow (9.43 Ha) in Lincolnshire, UK. The SRC willow field was planted with six closely related *Salix Viminalis* genotypes although here we targeted the genotype "Bjorn". Miscanthus and SRC willow were planted in 2006 and 2000 respectively on arable land which was previously managed on a rotation of 1 year oilseed rape followed by 3 years winter wheat. Miscanthus was planted at a density of 10,000 rhizomes ha^{-1} whilst SRC willow was planted at a density of 15,000 stools ha^{-1}. The soil was a fine loam over clay with approximately 53% sand, 32% silt and 15% clay (Case et al., 2012). Mean annual rainfall at the site was 605 mm (Scampton — approximately 3 km away) over 25 years from 1963 to 2004 with a mean annual minimum and maximum temperature of 5.9 °C and 13.1 °C respectively (1971–2000) (Drewer et al., 2012). The experiment was conducted in August 2012 with sampling continuing up to March 2013. In August 2012, rainfall was 67.5 mm with a mean air temperature of 16.28 °C ($\pm$3.8 SD). 2012 saw exceptional rainfall across the UK and locally the highest annual rainfall total since records began in 1947 (865.5 mm) (Waddington Met Office Monitoring Station — approximately 14 km away) (Barnes et al., 2017 manuscript in preparation). Both plantations were monitored by eddy covariance throughout the duration of the experiment using Li - Cor LI-7500 A open path IRGA's and Gill R3 Sonic Anemometers. For more detail of site soil parameters, eddy flux instrumentation and site characteristics see (Drewer et al., 2012).

2.2. Pre - pulse measurements

In order to obtain natural abundance δ^{13}C signatures for each pool, tissue samples of leaves and stems, bulk soil and roots were collected from the 6×2.5 m experimental plots described in section 2.3, one week before ^{13}C labelling. 5 plants were sampled in a transect across each plot and pooled into one composite sample of leaves and one of stems per plot. Bulk soil was sampled as described in section 2.7. Briefly, 3 cores were taken from each experimental plot, sectioned into 3 depths (0–10 cm, 10–20 cm and 20–30 cm)

and pooled to yield one composite sample per depth increment, per plot. Live roots were picked from the bulk soil samples. Plant tissues and bulk soil was measured for $\delta^{13}C$ as described in section 2.8. Soil temperature was measured using a digital thermometer probe at each permanent soil respiration collar. Soil moisture was measured in triplicate using an ML2 handheld theta probe (Delta-T Devices Ltd, UK) around the same collars. Soil respiration was measured as described in section 2.5. Leaf area index (LAI) was measured along a transect at the field scale using a LI - COR LAI - 2000 plant canopy analyser (LI-COR Biosciences, USA) and crop height was manually measured within each experimental plot. Total C and N of bulk soil samples was measured on a Costech ECS - 4010 Elemental Analyser (Costech Analytical Technologies Inc. CA, USA).

2.3. ^{13}C − pulse - chase labelling experiment

The miscanthus and SRC willow experimental plots were ^{13}C - labelled on August 23rd, 2012. Four experimental plots of 15 m^2 (6 m × 2.5 m) were established within each plantation. A pulse chamber was placed over the entire experimental plot using 10 mm diameter aluminium scaffolding up to a height of 3 m yielding a chamber volume of 45 m^3 (6 m × 2.5 m x 3 m). This design allowed for the inclusion within the 2.5 m, of two double rows of SRC willow planted 1.5 m apart. Clear polythene sheeting which allowed 90% of photosynthetically active radiation (PAR) to penetrate, was placed over the frame and the chamber sealed with rows of sandbags around the base. One 6.5 Kw water cooled, split air conditioning unit (Andrew Sykes, UK) and 2 tripod mounted fans were installed in each pulse chamber in order to counter ambient temperature increases and ensure homogeneous distribution of $^{13}CO_2$. Each pulse chamber was powered by a petrol generator. Pulse labelling commenced at ca. 08:20am. A total of 17 L of 99 atom % pure $^{13}CO_2$ (CK Gases, UK) was introduced to the tents at atmospheric pressure (within 3 L Tedlar bags), via a length of polythene flexible tubing. This was added in 3 sequential batches over a period of ca. 3 hrs in order to counter an above ambient CO_2 concentration increase. Disregarding plant uptake, the addition of 17 L of 99 atom % $^{13}CO_2$ would have equated to a 378 ppm increase in CO_2 concentration within the tent volume. However, plant uptake outstripped additional CO_2 supply leading to sub ambient CO_2 concentrations within the pulse tents, for the majority of the pulse labelling period (Supplementary Information − Fig. S3). Tents were left sealed for approximately 4.5 h when the chambers were removed. Soil respiration (Section 2.5), plant biomass (Section 2.6) and soils (Section 2.7) were sampled 1 week before and 4 h, 1, 2, 4, 7, 14, 21, 28, 42, 76, 104 & 194 days after labelling.

2.4. ^{13}C real − time monitoring

During pulse labelling, ^{13}C enrichment and CO_2 concentration was approximated within individual tents using a mobile lab fitted with a continuous flow Picarro G-2131i cavity ring down spectroscopy (CRDS) isotopic analyser (Picarro Inc. USA). We did not attempt to use this data to calculate the rate of ^{13}C uptake as ^{13}C concentrations immediately after $^{13}CO_2$ addition were far above the instruments calibrated range. However, it was used to monitor the drawdown of CO_2 during the enclosure period in order to determine when to start ^{13}C labelling and time the sequential additions of $^{13}CO_2$. A custom made vacuum - manifold system was used to draw air through 1/8" ID PTFE sampling lines from all 8 tents and 2 ambient air lines at a rate of 300 ml/min. Gas lines were sequentially sampled using a distribution manifold (Picarro Inc. USA) for 3 min increments per tent (supplementary information - Fig. S3). Unfortunately one miscanthus gas line was found to be leaking therefore these data were discarded.

2.5. Gas sampling & analysis

Two PVC chamber collars were permanently installed within the experimental area to 2 cm depth to minimise fine root and mycorrhizal disturbance (Heinemeyer et al., 2011; Mills et al., 2011). The chamber lid had a height of 20 cm and an internal diameter of 39 cm with an internal headspace volume of 0.03 m^2. 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 et al. (1995) at 0, 15, 30 and 45 min (post enclosure) and injected into 12 ml gas tight borosilicate glass vials (Labco, Lampeter, UK). Gas samples were analysed separately for CO_2 concentration and $\delta^{13}C$. A 10 ml sub - sample 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 (Picarro Inc. USA) feeding into a Small Sample Inlet Module (Picarro Inc, USA) and a Picarro G-2131i CRDS isotopic analyser. Linearity was checked by running 3 reference gases in triplicate (−9.98‰ at 414 ppm, −32.60‰ at 496 ppm, −36.51‰ at 1063 ppm) (BOC Gases, UK) whilst one reference gas sample (414 ppm, −9.98‰) was run after every 8 samples to account for linear drift and results were calibrated against these. 5 ml of the remaining field sample was transferred to a 3 ml evacuated 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 Detector (FID) operating at 130 °C. The GC was fitted with a stainless steel Porapak Q 50−80 mesh column (length 2 m, outer diameter 3.17 mm) maintained at 60 °C. Three calibration gas standards (500 ppm, 1000 ppm, 4000 ppm CO_2) (Air Products, Waltham on Thames, UK) were run every 14 samples (Case et al., 2012).

2.6. Biomass sampling

Aboveground biomass samples were collected at both miscanthus and SRC willow experimental plots. Fresh leaves and stems were collected from both upper and lower sections of 5 plants across the plots, transferred to labelled bags and immediately stored on ice in a cool box. No SRC willow leaves were collected at 76, 104 & 194 days due to senescence. Samples were subsequently frozen (−20 °C) within 2 h of collection, before being oven dried at 60 °C and cryo - milled (SPEX SamplePrep, Freezer/Mill 6770) to a fine powder for isotopic analysis.

2.7. Soil sampling

Soil samples were obtained with a 2.5 cm diameter gouge auger (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands). Three cores were taken within the experimental area and sectioned into 3 depths; 0−10, 10−20 & 20−30 cm. These sections were pooled to give a total of 3 samples per experimental plot; one at each depth increment. Soils were frozen at −20 °C within 2 h of collection. These were subsequently freeze - dried (Christ alpha 1−4 LD Plus) and then sieved to 2 mm. Stones were removed while roots were picked out and transferred to glass vials. The remaining soil was ball milled (Fritsch Planetary Mill Pulviresette 5) to a fine powder. Picked roots were washed, oven dried at 60 °C and cryo - milled (SPEX SamplePrep, Freezer/Mill 6770) to a fine powder.

2.8. Bulk ^{13}C analysis

Subsamples of the ground bulk soil, roots and plant biomass were analysed using a Costech ECS - 4010 Elemental Analyser

(Costech Analytical Technologies Inc. CA, USA) coupled to a CRDS Picarro G-2131i isotopic analyser (Picarro Inc. CA, USA) via a split - flow interface using a method similar to (Balslev-Clausen et al., 2013). Combustion gases were vented through a Picarro Caddy split - flow interface before passing into the isotopic analyser for $\delta^{13}C$ analysis. Cane sugar ($-11.64‰$) and beet sugar ($-26.03‰$) (purchased from Iso - Analytical, UK) were used as isotopic standards with a working standard error of ± 0.21 (SE) ‰. Natural abundances of ^{13}C are here expressed as $\delta^{13}C$ as defined by Equation (1) where $^{13}C/^{12}C_{PDB}$ is the isotopic ratio of the standard material PDB given as 0.0112372 and $^{13}C/^{12}C_{sample}$ is the isotopic ratio of a measured sample.

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

Enriched results were converted to atom % excess and standard flux equations were used to partition ^{12}C and ^{13}C and calculate the absolute amount of excess ^{13}C in soil respiration (Supplementary Information – S1).

2.9. PLFA and ^{13}C - PLFA analyses

Subsamples of the 0–10 and 10–20 cm freeze dried ground soil collected on day 1, 2, 4, 7, 14, 21, 28, 42, 76, 104 and 194 were bulked by sampling point and PLFA's extracted as part of the total lipid extract using a modified Bligh-Dyer extraction (White et al., 1979). For more detail of the extraction procedure and full QA/QC see supplementary information – S2. Identification of PLFA's was carried out on a GC (Agilent Technologies 6890) fitted with a mass selective detector (Agilent Technologies 5973). The terminal and mid - chain branched fatty acids C15:0i, C15:0a, C16:0i C17:0i and C17:0a were used as indicators of Gram positive bacteria (Whitaker et al., 2014). Cyclopropyl saturated and monounsaturated fatty acids 16:1ω7c, 7,8 cyclic C17:0, C18:1ω7c & 7,8 cy-C19:0 were used as indicators of Gram negative bacteria (Rinnan and Baath, 2009). The fatty acids C18:2ω6,9c and C18:1ω9c were taken as indicators of fungi (Kaiser et al., 2010). Total microbial biomass was taken as the sum of all identified PLFA's (n = 24) (C13:0, C14:0, C14:1ω5c, C15:0, C15:1ω5c, C16:0, 10Me-C16:0, C16:1ω7t, C16:1ω9c, C16:1ω5c, C17:0, 10Me-C17:0, C18:0i, C17:1ω7c, C18:0a, C18:0, 10Me-C18:0, C18:1ω7t, C18:1ω12c, C18:1ω5c, C18:2ω6t, 9,10-cy-C19:0, C19:1ω12c, C20:0, C18:3ω6c, C20:1ω9c, C18:3ω3c, C20:2ω6c, C22:0, C20:3ω6c, C20:4ω6c, C20:5ω3c, C24:0; plus those listed above). The PLFA 16:1ω5 has been proposed as a biomarker for AMF. However, it was not used here as it is also found in considerable quantities in Gram negative bacteria (Ngosong et al., 2012; Ruess and Chamberlain, 2010; Sharma and Buyer, 2015).

Individual PLFA's were analysed for $\delta^{13}C$ using GC - combustion - isotope ratio mass spectrometry (GC-C-IRMS) (Isoprime Ltd). The PLFA $\delta^{13}C$ values were corrected for the addition of the extra carbon atom introduced to the molecule during methylation, using a correction factor obtained by CF – EA - IRMS measurement on the derivatising methanol and application of the mass balance equation of Jones et al. (1991). For details of the instrumental set up see supplementary information – S2.

2.10. Turnover calculations of assimilated C

To investigate differences in the rate of labile C turnover through leaves, one - phase exponential decay functions were fitted to the first 7 time points, 4 h until 21 days after labelling for leaf biomass. The exponential decay was taken to represent the labile C pool (A) whilst the non - zero asymptote represented the fraction fixed either into structural biomass or long term storage pools (B) (Carbone et al., 2007; Shibistova et al., 2012; Studer et al., 2014;

Subke et al., 2012). These functions were used to estimate the mean residence time (MRT) of C in pool A and the size of pool B relative to maximum enrichment here defined as CUE. It is assumed that losses from pool A were due to phloem transport, respiration or incorporation into pool B rather than dilution due to fixation of unlabelled C. The exponential decay is represented by the following function (Equation (2)).

$$C(t) = P + C_0 \exp^{(-kt)} \qquad (2)$$

Where C(t) represents the amount of labelled C at time t, C_0 the amount of C at peak, t is the time after peak, k is the rate constant and P is the non-zero asymptote. The MRT was calculated by taking the inverse of the decay constant (b) (Equation (3)).

$$MRT = k^{(-1)} \qquad (3)$$

CUE was estimated by taking the parameter P, which is the y - intercept of the non - zero asymptote and represents labelled C remaining once decay is complete and dividing by the initial maximum enrichment (C_0) in atom % excess (Equation (4)).

$$CUE = P/C_0 \qquad (4)$$

This represents a simple approximation of the fraction of gross primary productivity fixed in leaf biomass only and not the whole plant.

2.11. Statistical analysis

All linear mixed effects models were performed using R statistical software (R Core Team, 2017). Assumptions of normality, homogeneity and independence were assessed graphically. In all but one of the linear mixed effects models, to account for the repeated measures design, the random structure was specified as random intercepts of plot. In the case of the $^{13}CO_2$ flux model, chamber was nested within plot to account for this pseudo-replication. Where multiple fixed effects were considered, the optimal fixed structure was then obtained using the top down approach to model selection as outlined in Zuur et al. (2009). P - values for fixed terms in linear mixed effects models were obtained by likelihood ratio tests of the model using maximum likelihood estimation, with the effect in question against the model without the effect in question. The likelihood ratio test statistics are presented with their associated χ^2 distribution with degrees of freedom reported in brackets. R^2 values for linear mixed effect models were calculated (Nakagawa and Schielzeth, 2013) using the r.squaredGLMM function in the MuMIn package (Barton, 2015). The nlme package (Pinheiro et al., 2015) was used to test for relationships between the following: 1. PLFA concentrations, land use and sampling season, 2. ^{13}C enrichment in aboveground biomass over time, 3. Root ^{13}C allocation between land uses, sampling depths and over time, 4. Bulk soil ^{13}C allocation between land uses, sampling depths and over time, 5. ^{13}C assimilation into fungal PLFA's between land uses and over time, 6. Percentage of total PLFA - ^{13}C allocated to fungal and bacterial PLFA between land uses over time, 7. Soil $^{13}CO_2$ efflux between land uses and over time. One - phase exponential decay models were created in GraphPad Prism (GraphPad Software 7.02, La Jolla California USA) using the non - linear regression function. To test whether fitted parameters were different between crops the extra sum of squares F test was used where separate best - fit values were fitted for the rate constant (k) and non - zero asymptote (P) vs. a model where those parameters were shared among data sets. For detailed descriptions of the statistical models used refer to supplementary information – S3 and Tables S2 – S6.

Table 1
Mean PLFA concentrations (µg PLFA g^{-1} soil dry weight) from soil sampled between 0 and 20 cm depth. Summer represents the average of sampling on days 0, 1, 2, 4 & 7. Autumn represents the average of sampling on days 14, 21, 28, 42 & 76. Winter & Spring represent sampling on day 104 and 194 after the pulse respectively. Mean represents the average of all sampling points (n = 12). Gram Positive (G+) - (C15:0i, C15:0a, C16:0i C17:0i & C17:0a), Gram Negative (G-) - (16:1ω7c, 7,8 cyclic C17:0, C18:1ω7c & 7,8 cy-C19:0), Fungal - (C18:2ω6,9c and C18:1ω9c), F:B - Fungal to Bacterial Ratio, GP:GN - Gram Positive to Gram Negative Ratio. Data are means (±1 SE) (n = 4).

| Season	Land Use	Total PLFA	Fungal PLFA	Bacterial PLFA	G + PLFA	G- PLFA	F:B PLFA	GP:GN PLFA
Summer	Miscanthus	30.2 (2.6)	2.4 (0.3)	15.2 (1.4)	7.0 (0.5)	8.3 (0.9)	0.15 (0.01)	0.86 (0.04)
	SRC Willow	48.4 (5.3)	6.5 (1.0)	22.4 (2.3)	9.7 (1.0)	12.7 (1.4)	0.29 (0.02)	0.77 (0.02)
Autumn	Miscanthus	31.7 (2.3)	2.9 (0.3)	16.0 (1.2)	7.1 (0.5)	8.9 (0.7)	0.18 (0.01)	0.79 (0.02)
	SRC Willow	47.2 (6.8)	6.0 (1.1)	22.5 (3.0)	9.7 (1.1)	12.8 (1.9)	0.26 (0.02)	0.77 (0.03)
Winter	Miscanthus	30.3 (2.7)	2.9 (0.4)	15.7 (1.4)	6.8 (0.5)	8.9 (0.9)	0.18 (0.01)	0.78 (0.02)
	SRC Willow	51.8 (5.3)	6.3 (1.0)	25.9 (2.4)	10.8 (0.9)	15.1 (1.6)	0.24 (0.01)	0.72 (0.03)
Spring	Miscanthus	31.8 (0.5)	3.0 (0.1)	16.7 (0.3)	7.2 (0.1)	9.5 (0.2)	0.18 (0.00)	0.76 (0.02)
	SRC Willow	42.7 (4.2)	4.8 (0.7)	21.4 (1.8)	9.0 (0.7)	12.4 (1.1)	0.22 (0.02)	0.73 (0.02)
Mean	Miscanthus	30.9 (0.7)	2.7 (0.1)	15.7 (0.4)	7.0 (0.2)	8.7 (0.2)	0.17 (0.00)	0.82 (0.01)
	SRC Willow	47.7 (1.7)	6.2 (0.3)	22.7 (0.7)	9.7 (0.3)	12.9 (0.5)	0.27 (0.01)	0.76 (0.01)

3. Results

3.1. Microbial community structure and total PLFA's

Higher total PLFA concentrations were found under SRC willow relative to miscanthus (Table 1) (χ^2 (1) = 7.56, p = 0.006, R^2_c = 0.92) with concentrations of bacterial PLFA (χ^2 (1) = 6.85, p = 0.009, R^2_c = 0.88), Gram positive PLFA (χ^2 (1) = 7.18, p = 0.007, R^2_c = 0.88) and Gram negative PLFA (χ^2 (1) = 6.60, p = 0.010, R^2_c = 0.88) all following this trend (Table 1). An interaction between season and crop showed that the relationship between fungal PLFA abundance and crops was dependent upon time sampled with fungal abundance declining over time under SRC willow relative to a slight increase under miscanthus (χ^2 (3) = 13.05, p = 0.005, R^2_c = 0.97). However, over the measurement period, fungal PLFA concentrations were 3.12 (±0.99 SE) (µg PLFA g^{-1} soil dry weight) higher under SRC willow relative to miscanthus. Of the total PLFA concentration in soils, 47% and 51% were assigned to bacterial biomarkers (bacterial PLFA concentration relative to total PLFA concentration) with 13% and 9% to fungal biomarkers under SRC willow and miscanthus respectively (Fig. 1). Fungal to bacterial

(F:B) ratios mirrored fungal abundance with ratios declining from 0.29 (±0.02 SE) to 0.22 (±0.02 SE) during the sampling period but under miscanthus F:B ratios increased from 0.15 (±0.01 SE) to 0.18 (±0.00 SE). This was highlighted by an interaction between season and crop (χ^2 (3) = 19.56, p = <0.001, R^2_c = 0.95).

3.2. ^{13}C assimilation and recovery in foliage and stems

Pre - labelling natural abundance $\delta^{13}C$ measurements for miscanthus and SRC willow tissues were typical of C4 and C3 plant communities (Boutton, 1991) (Table 2). On the day of the experiment, mean gross primary productivity (GPP) was higher for miscanthus relative to SRC willow (Table 2). Above ground tissues (stems & leaves) in both crops were enriched above natural abundance (natural abundance = 0) 4 h after labelling with average enrichments of 0.11 atom % excess (0.08–0.15 95% Confidence Interval (CI)) for miscanthus and 0.15 atom % excess (0.07–0.22 95% CI) for SRC willow. Time lag to peak enrichment was 4 h in the leaves of both crops, 48 h in SRC willow stems, 24 h in miscanthus lower stems and 48 h in miscanthus upper stems (Fig. 2). Over the sampling period, ^{13}C enrichment declined towards the natural
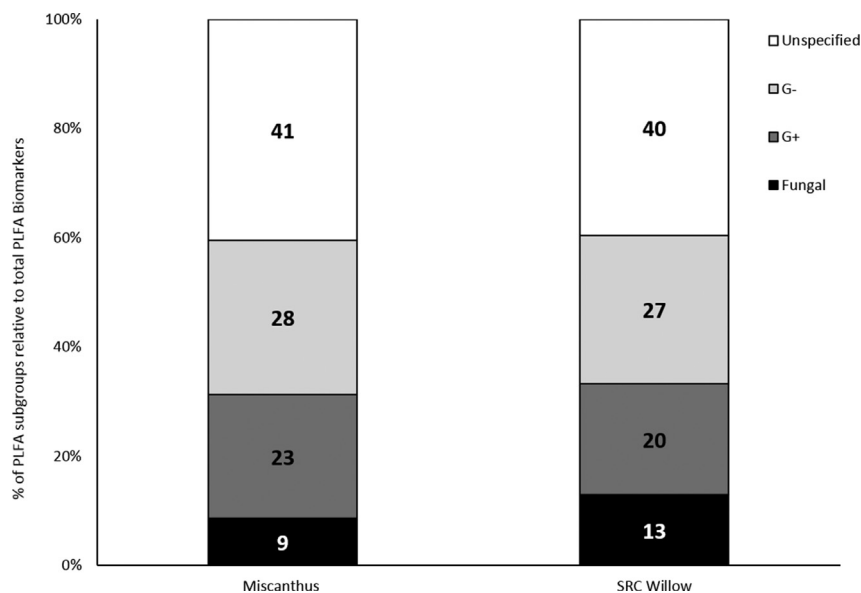

Fig. 1. Relative microbial community structure determined from % of PLFA assigned to functional groups relative to total PLFA in Miscanthus and SRC willow soils. Gram Positive (G+) - (C15:0i, C15:0a, C16:0i C17:0i & C17:0a), Gram Negative (G-) - (16:1ω7c, 7,8 cyclic C17:0, C18:1ω7c & 7,8 cy-C19:0), Fungal - (C18:2ω6,9c and C18:1ω9c), Unspecified (non-specific) − (C13:0, C14:0, C14:1ω5c, C15:0, C15:1ω5c, C16:0, 10Me-C16:0, C16:1ω7t, C16:1ω9c, C16:1ω5c, C17:0, 10Me-C17:0, C18:0i, C17:1ω7c, C18:0a, C18:0, 10Me-C18:0, C18:1ω7t, C18:1ω12c, C18:1ω5c, C18:2ω6t, 9,10-cy-C19:0, C19:1ω12c, C20:0, C18:3ω6c, C20:1ω9c, C18:3ω3c, C20:2ω6c, C22:0, C20:3ω6c, C20:4ω6c, C20:5ω3c, C24:0). Data represents mean of 12 time points; 0,1,2,4,7,14,21,28,42,76,104,194 days after labelling.

Table 2
Pre pulse plot measurements of soil properties, crop parameters, natural abundance isotopic signatures of above and belowground vegetation and bulk soil and eddy flux measurements. LAI data are means ± 1 Standard Deviation. All other measures are means ± 1 standard error. Soil properties and natural abundance $\delta^{13}C$ (n = 4), soil moisture (n = 24), soil temperature and CO_2 flux (n = 8).

Pre - Pulse Measurements	Miscanthus	SRC Willow
Crop Height (m)	2.1	4.3
Bulk Density (g/cm^3)	1.38 (0.05)	1.36 (0.04)
LAI	3.3 (0.6)	2.2 (0.3)
% N (0−30 cm)	0.18 (0.01)	0.19 (0.01)
% C (0−30 cm)	1.90 (0.08)	2.26 (0.17)
Soil Moisture (%)	27.5 (1.1)	26.8 (0.8)
Soil Temperature (%)	19.1 (0.2)	18.8 (0.3)
CO_2 Flux (mg CO_2 - C m^2 h^{-1})	101.35 (13.31)	105.70 (13.74)
Natural Abundance ($\delta^{13}C$)		
Stems ($\delta^{13}C$)	−11.81 (0.09)	−28.56 (0.28)
Leaves ($\delta^{13}C$)	−11.84 (0.08)	−29.66 (0.13)
Roots ($\delta^{13}C$)	−13.84 (0.38)	−29.70 (0.21)
Soil ($\delta^{13}C$)	−25.52 (0.32)	−26.63 (0.20)
Eddy Flux Measurements		
Annual Monthly Peak GPP (μmol m^2 s^{-1})	August 13.32 (0.41)	July 11.96 (0.32)
GPP (Pulse Day) (μmol m^2 s^{-1})	13.72 (2.26)	10.59 (1.86)
Air Temp (°C) (Pulse Day)	14.84 (0.41)	14.88 (0.41)
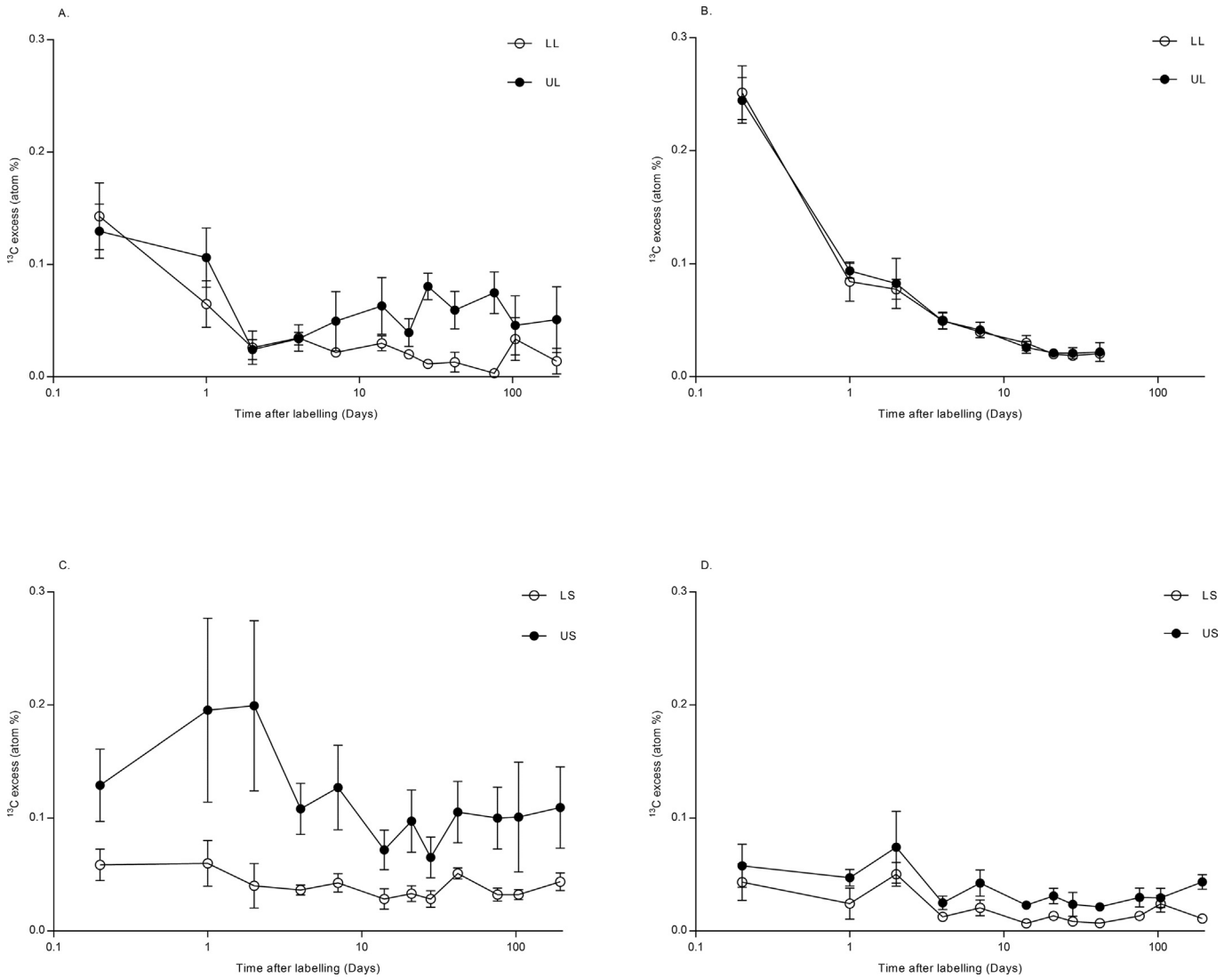
Fig. 2. Time course of pulse derived ^{13}C allocation in above - ground biomass partitioned into upper leaves (UL), upper stems (US), lower leaves (LL) and lower stems (LS). A. - Miscanthus leaf, B. − SRC Willow leaf, C. − Miscanthus stem, D. − SRC Willow stem. Error bars represent ±1 SE (n = 4). Note: x-axis is plotted logarithmically.

abundance isotopic signature. Senescence of willow leaves occurred between 42 and 76 days after labelling, at which point no more leaf samples were taken. All other tissues retained labelled C after 194 days (Fig. 2). Over the sampling period, ^{13}C enrichment in miscanthus was greater in stems relative to leaves (χ^2 (1) = 39.15, p = <0.001, R^2_c = 0.71) and in samples taken from upper relative to lower positions (χ^2 (1) = 65.30, p = <0.001, R^2_c = 0.71). In SRC willow, an interaction between sampling position and plant tissue showed that whilst in the stems there was a greater enrichment in upper compared to lower sampling positions, there was no difference between upper and lower leaves (χ^2(1) = 12.74, p = <0.001, R^2_c = 0.45). Based on the best model fit (one - phase exponential decay) (Fig. 3A. − 3D.), there was no difference in the MRT of the labile C pool between miscanthus and SRC willow (Table 3) ($F_{3,102}$ = 0.18, p = 0.91). The CUE was higher in miscanthus upper leaves relative to SRC willow ($F_{1,52}$ = 10.11, p = 0.0025) although there was no difference between lower leaves ($F_{1,52}$ = 0.07, p = 0.80).

3.3. ^{13}C translocation belowground

Roots were elevated above natural abundance levels in both crops 4 h after labelling, with average enrichments of

0.0014 ± 0.0005 (SE) and 0.0011 ± 0.0005 (SE) ^{13}C atom % excess in miscanthus and SRC willow roots respectively (Fig. 4). Over the study period, there was no difference in ^{13}C enrichment between crops (χ^2 (1) = 2.18, p = 0.14, R^2_c = 0.80). Miscanthus rhizomes were also enriched above natural abundance. However, high between plot variability confounded interpretation (supplementary information − Fig. S1). For bulk soil, over the study period there was no difference in the ^{13}C enrichment between the crops (χ^2 (1) = 0.30, p = 0.58, R^2_c = 0.09).

3.4. ^{13}C recovery in microbial PLFA's

24 h after labelling allocation of pulse derived ^{13}C to PLFA's was detected in both miscanthus (total PLFA - 0.60 ± 0.15 (SE) ng PLFA − ^{13}C g^{-1} dry weight soil) and SRC willow. (total PLFA − 1.30 ± 0.33 (SE) ng PLFA − ^{13}C g^{-1} dry weight soil). Fungal PLFA were more enriched under SRC willow relative to miscanthus over the sampling period (χ^2 (1) = 13.96, p = <0.001, R^2_c = 0.51). Time lag to peak enrichment in fungal PLFA was 4 and 7 days for the miscanthus (1.47 ± 0.75 (SE) ng PLFA − ^{13}C g^{-1} dry weight soil) and SRC willow (1.62 ± 0.42 (SE) ng PLFA − ^{13}C g^{-1} dry weight soil) respectively, although the peak was far more pronounced under miscanthus. Time lag to peak enrichment in bacterial PLFA was
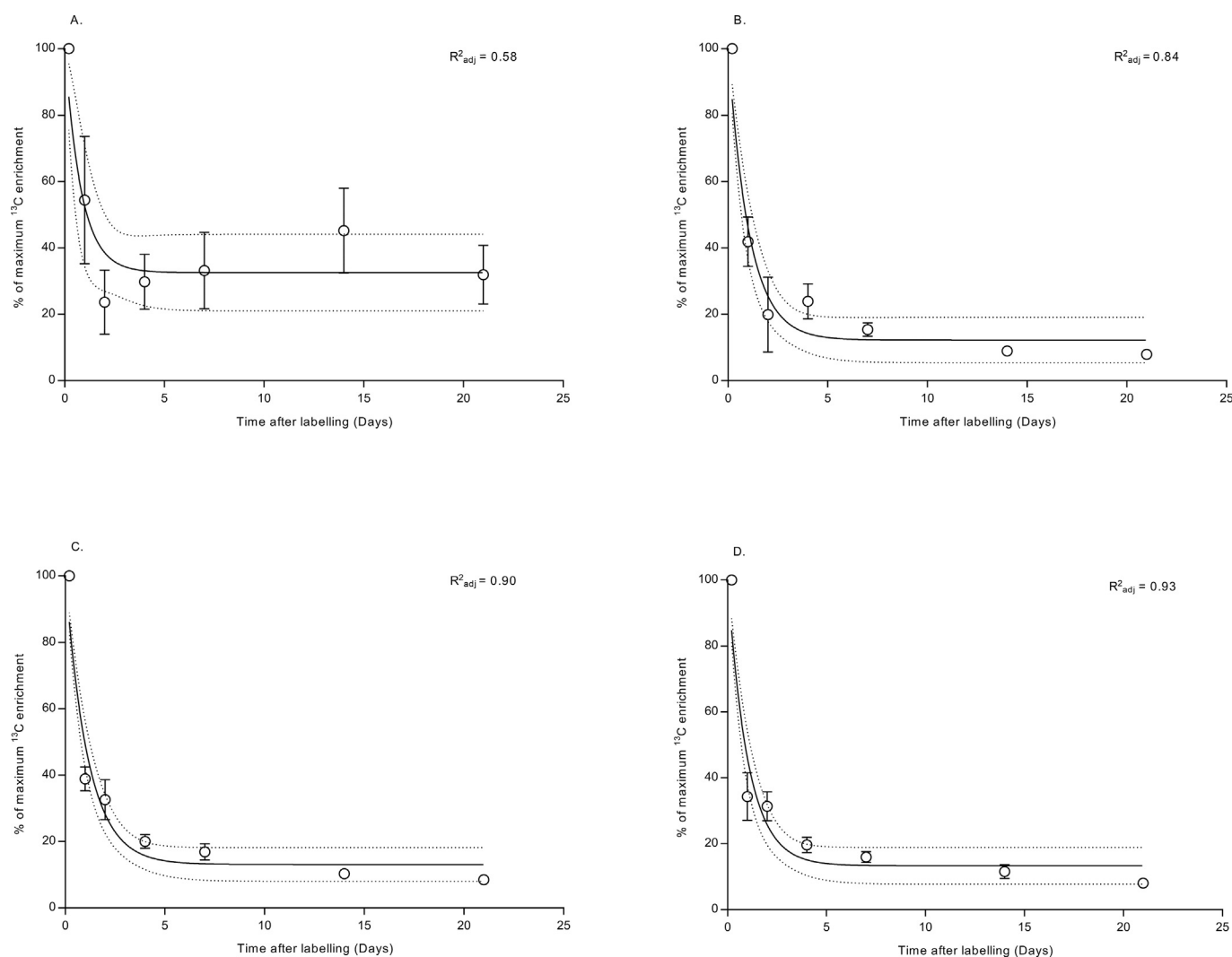

Fig. 3. Fitted one - phase exponential decay functions representing the decline of labile ^{13}C within leaves over the first 7 time points up until 21 days after labelling. Data is plotted as a percentage of peak enrichment (peak = 100%). A. = Miscanthus upper leaf, B. = Miscanthus lower leaf, C. = SRC willow upper leaf, D. = SRC willow lower leaf. Dashed lines represents 95% confidence intervals.

Table 3
Half - lives, mean residence times (MRT) and carbon use efficiency (CUE) of assimilated ^{13}C in leaf biomass. These were calculated from fitted one - phase exponential decay functions fitted to data in Fig. 3. Brackets represent 95% confidence intervals. CUE calculated as the % of enrichment remaining (non - zero asymptote) relative to maximum enrichment.

Crop	Tissue	Half Life (Days)	MRT (Days)	CUE (%)
Miscanthus	Upper Leaf	0.72 (0.38–1.28)	1.04 (0.54–1.85)	32.75 (22.26–42.75)
Miscanthus	Lower Leaf	0.73 (0.51–1.03)	1.06 (0.74–1.49)	12.22 (5.25–18.89)
SRC Willow	Upper Leaf	0.79 (0.60–1.03)	1.15 (0.87–1.49)	13.09 (7.83–18.17)
SRC Willow	Lower Leaf	0.86 (0.68–1.08)	1.24 (0.98–1.56)	12.51 (7.97–16.91)
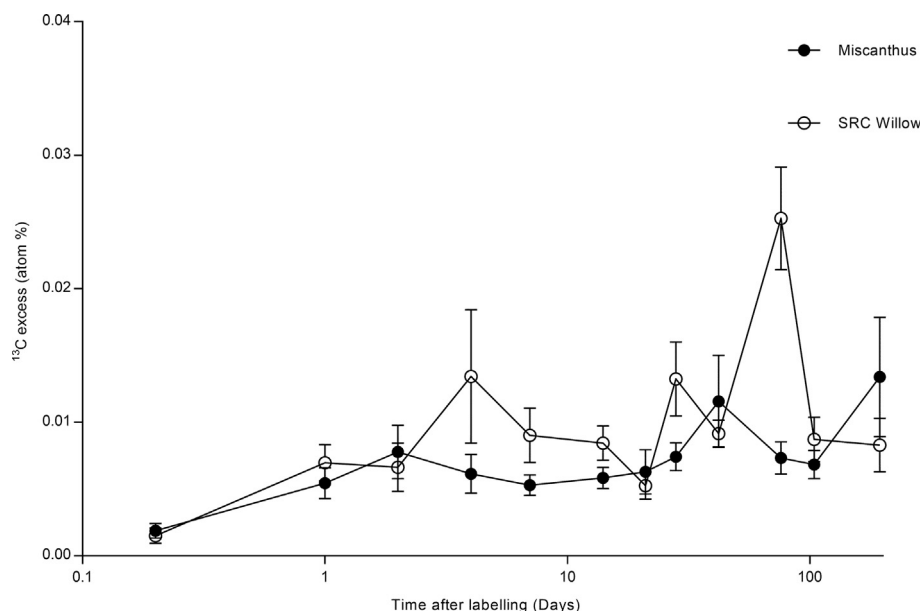
Fig. 4. Time course of pulse derived ^{13}C allocation into roots. Data represent the mean of samples taken from miscanthus and SRC willow at 0–10 cm, 10–20 cm and 20–30 cm depths (n = 12). Error bars represent ±1 SE. Note X - axis is plotted logarithmically.

again detected 4 days after labelling for miscanthus, (8.46 ± 5.22 (SE) ng PLFA − ^{13}C g^{-1} dry weight soil), whilst SRC willow enrichment was generally low (<0.8 ng PLFA − ^{13}C g^{-1} dry weight soil) with no clear peak (Fig. 5). Over the sampling period, bacterial PLFA held a greater % of total pulse derived PLFA - ^{13}C under miscanthus (52%) relative to SRC willow (23%) (χ^2 (1) = 27.66, p = <0.001, R^2_c = 0.79). An interaction between the % of total pulse derived PLFA - ^{13}C in fungal PLFA and time after labelling, highlighted that the proportion of ^{13}C enrichment in fungal PLFA increased over time under SRC willow and decreased over time under miscanthus (χ^2 (1) = 10.60, p = <0.001, R^2_c = 0.83). Over all timepoints, fungal PLFA held a greater % of total pulse derived ^{13}C under SRC willow (44%) relative to miscanthus (9%) (Fig. 6).

3.5. ^{13}C recovery in soil CO_2 efflux

^{13}C flux in soil respiration was higher under SRC willow relative to miscanthus over the first 14 days (χ^2 (1) = 13.61, p = <0.001, R^2_c = 0.64) (Fig. 7). The greatest excess flux of ^{13}C in soil respiration was detected 48 h after labelling under SRC willow (260.6 ± 67.9 (SE) mg CO_2-^{13}C m^{-2} h^{-1}) and miscanthus (40.2 ± 9.6 (SE) mg CO_2-^{13}C m^{-2} h^{-1}). Overall 380% more ^{13}C was returned to the atmosphere in soil respiration from under SRC willow during the sampling period relative to miscanthus (Fig. 7). The difference was particularly marked during the first 7 days post labelling where ^{13}C excess flux under SRC willow was at least 5 times greater than under miscanthus. ^{13}C excess in soil respiration declined over the 194 day sampling period with 92% of total respired ^{13}C lost from

under SRC willow in just the first 7 days relative to 72% under miscanthus.

4. Discussion

### 4.1. Assimilation of C into plant biomass

The MRT of ^{13}C in the labile C pool (A) was similar between the two crops, irrespective of sampling position at around 1 day. This is surprising as previous studies have shown that the leaf vascular system of C4 plants supports a superior photosynthate translocation and distribution system relative to C3 plants (McKown and Dengler, 2009; Ueno et al., 2006). In comparison to other studies performed on different species, this MRT is shorter than those generally observed on trees (Hogberg et al., 2008; Ruehr et al., 2009; Warren et al., 2012) but more comparable to those observed on shorter vegetation such as grasses, shrubs and tundra vegetation (Carbone and Trumbore, 2007; Subke et al., 2012). This highlights the rapid translocation of assimilates from leaves in both crops.

We also found no differences in the transfer rates of labelled assimilates from leaves into other plant tissues above and belowground between miscanthus (C4) and SRC willow (C3). Translocation of C from leaves to stems, roots and into soil respiration occurred before our first measurement, 4 h after labelling. This is contradictory to our hypothesis that miscanthus would translocate C belowground more quickly than SRC willow and is surprising given the height of the vegetation. The rate of transfer from photosynthetic biomass to soil respiration is dependent mainly
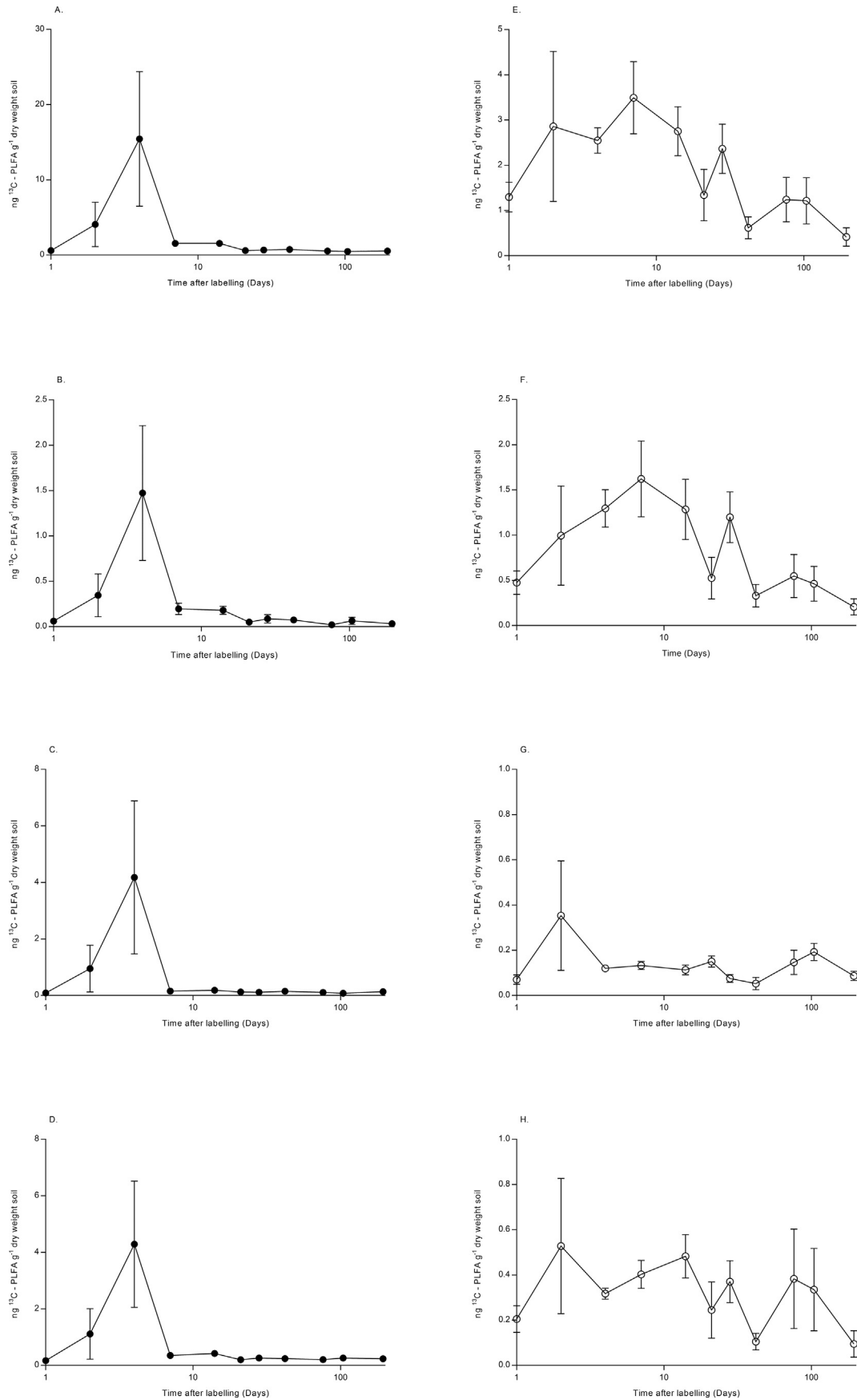
Fig. 5. Time course of pulse derived ^{13}C incorporation into PLFA. Miscanthus A - D, SRC Willow E − H. (A,E) = Total PLFA, (B,F) = Fungal PLFA, (C,G) = Gram positive PLFA & (D,H) = Gram negative PLFA. Error bars represent ±1 SE (n = 4). Note that x-axis is plotted logarithmically and y - axis have different scales.
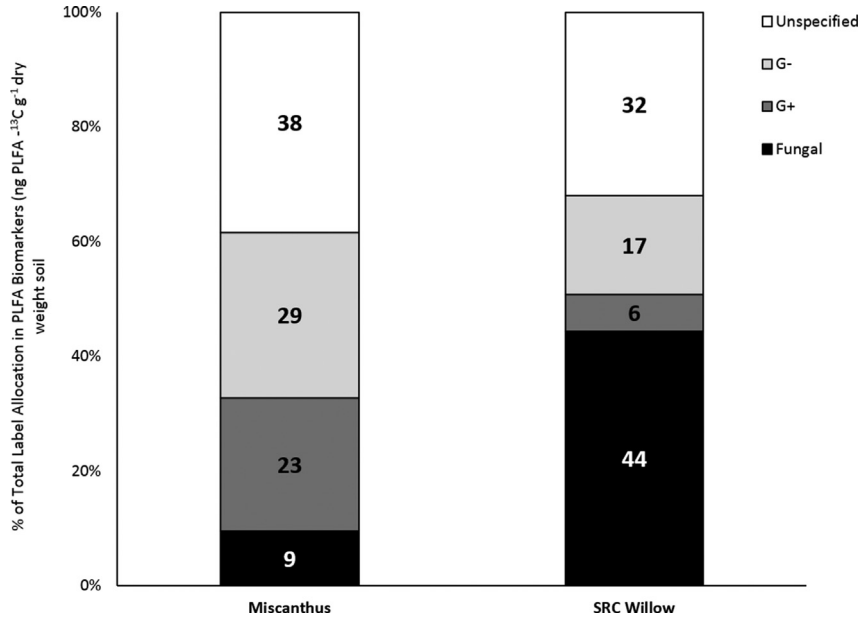
Fig. 6. The % of pulse derived ^{13}C detected in fungal, Gram positive (G+), Gram negative (G-) and unspecified (non - specific) microbial PLFA relative to the total pulse derived ^{13}C detected in all PLFA across the study period. Data represents the mean of timepoints 1, 2, 4, 7, 14, 21, 28, 42, 76, 104 and 194 days. Standard Errors ($\pm$1 SE) are as follows: Miscanthus % Fungal (0.72), % G+ (1.34), % G- (1.85) % Unspecified (2.12). SRC Willow % Fungal (1.70), % G+ (1.31), % G- (0.96) % Unspecified (1.86).

upon path length as well as phloem transport velocity and environmental conditions (Dannoura et al., 2011; Kuzyakov and Gavrichkova, 2010). With respect to SRC willow, this time lag is faster than has been observed on 3 m tall hybrid poplars (12 h) and mature trees (4–5 days) (Horwath et al., 1994; Kuzyakov and Gavrichkova, 2010). Coppicing may influence the phloem transport velocity of C as poplars have been shown to respond to coppicing by increasing belowground C allocation (Berhongaray et al., 2015). Whilst no other labelling studies have been performed on miscanthus, the time lag measured here is comparable to that measured on perennial reed canary grass (5 h) (Tavi et al., 2013).

In partial agreement with our first hypothesis, we observed a higher CUE in miscanthus upper leaves but not in lower leaves relative to SRC willow. Moreover, ^{13}C enrichment was higher in upper relative to lower miscanthus stems however, no partitioning was seen in SRC willow leaves and stems. These findings suggest that in miscanthus, a greater proportion of labelled assimilates were preferentially allocated toward the synthesis of new photosynthetic biomass and supporting structures. These results may reflect differences in the phenological stage of the two crops rather than differences in their physiology as Desalme et al. (2017) also showed C partitioning to new photosynthetic biomass in C3 trees early in their growing seasons. This appeared to be confirmed by
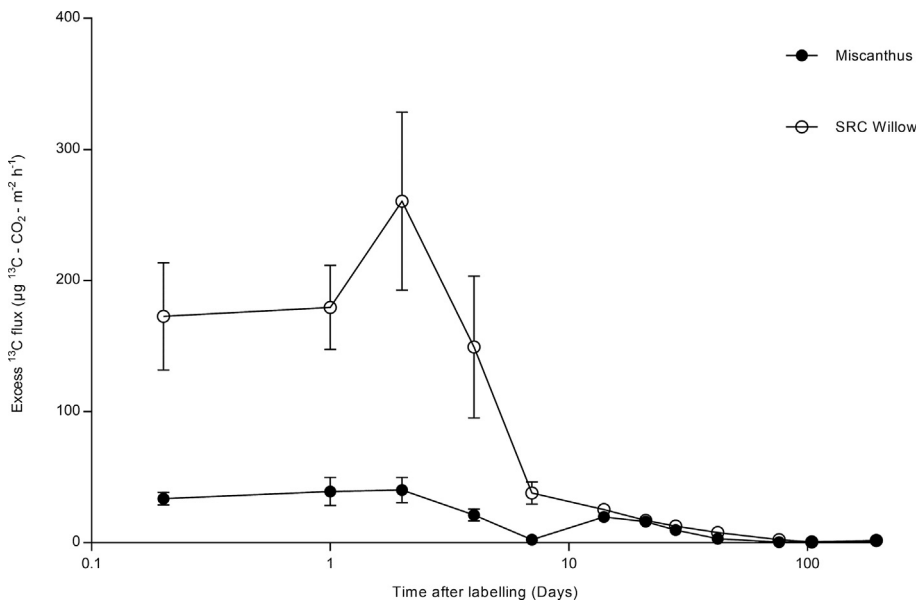
Fig. 7. Excess ^{13}C flux in soil respiration from miscanthus and SRC willow plots across 194 day sampling period. Data are means and error bars represent $\pm$1 SE (n = 8). Note. X - axis is plotted logarithmically.

eddy covariance which showed that annual mean monthly gross primary productivity (GPP) had peaked in July for SRC Willow whilst miscanthus was at peak growth in August (Table 2). Moreover, on the day of the experiment GPP was higher for the miscanthus than for SRC willow (Table 2). The higher proportion of ^{13}C fixed into miscanthus leaf biomass (CUE) seemed to be reflected in differences in the magnitude of belowground C transport, with a lower ^{13}CO$_2$ flux measured in soil respiration, relative to SRC willow. However, no differences were observed in root ^{13}C enrichments over the measurement period.

4.2. Carbon transfer to soil microbial communities

PLFA's are essential components of all living cells, are not found in storage products or necromass, and have been used to profile microbial functional groups in soils (Zelles, 1999). This method has low phylogenetic resolution in comparison to DNA based approaches but is useful in identifying active microbial populations through stable isotope probing (Garcia-Pausas and Paterson, 2011; Gutierrez-Zamora and Manefield, 2010; Helfrich et al., 2015; Neufeld et al., 2007b, 2007a; Tavi et al., 2013). Land use change has been shown to alter soil microbial community composition (French et al., 2017; Tosi et al., 2016; Zhang et al., 2016). We found higher total PLFA concentrations under SRC Willow relative to miscanthus (Table 1). This suggests greater microbial biomass under SRC willow as total PLFA concentrations have been used as a proxy for total microbial biomass (Bååth and Anderson, 2003; Leckie et al., 2004). We also found higher fungal to bacterial ratios under SRC willow relative to miscanthus (Table 1). The fungal biomarkers used here (C18:2ω6,9c and C18:1ω9c) have been correlated with ergosterol, another fungal biomarker (Högberg, 2006; Klamer and Bååth, 2004) with a negligible contribution from roots (Kaiser et al., 2010). This suggests greater fungal dominance under SRC willow relative to miscanthus. However, these biomarkers are present in EM mycelium as well as saprotrophic fungi (Kaiser et al., 2010). Therefore, observed differences may also reflect differences in the crops respective symbionts. Both adjacent sites share a common management history and these distinctions in microbial PLFA concentrations are presumed to reflect changes to the current vegetation cover. A reduction in the F:B ratio in SRC willow was observed across the sampling period coinciding with the end of the growing season and senescence. Willows can potentially be colonized by AMF or EMF (Becklin et al., 2012; Corredor et al., 2014) and it has been shown that the EMF mycelial system is strongly dependent on current assimilates (Hogberg et al., 2001). Therefore the observed reduction in the F:B ratio under SRC willow may be attributable to a decrease in fungal biomass as a result of reduced below - ground photosynthate supply (Ellstrom et al., 2015; Hogberg et al., 2001).

It has been suggested that "niche differentiation" exists between fungal and bacterial decomposers with respect to the decomposition of SOM, where bacteria and fungi predominate in the decomposition of simple and recalcitrant compounds respectively (Boer et al., 2005). We observed higher ^{13}C concentrations in Gram positive and Gram negative biomarkers relative to fungal under miscanthus. This supports the niche theory and suggests that bacteria were more active in cycling recent and likely simple plant assimilates within the rhizosphere, under the miscanthus plots. Indeed, other studies have shown rapid allocation of C to rhizosphere inhabiting bacteria (Vandenkoornhuyse et al., 2007), with Gram negative bacteria particularly active in the cycling of labile C compounds, such as exudates in the rhizosphere (Bird et al., 2011; Koranda et al., 2014; Treonis et al., 2004). However in our experimental plots, no differences were seen in the speed or magnitude of ^{13}C

incorporation between Gram positive and Gram negative bacteria suggesting no functional differentiation. In the SRC willow ^{13}C was concentrated predominantly into fungal biomarkers with peak enrichment after 7 days. Parallel fungal community sequence analysis in summer 2010 revealed that this SRC willow crop only supported EMF fungi with the most abundant root associated fungal taxa being the EMF *Sebacina* spp and *Cortinarius* spp (Barnes et al., 2016). The high allocation to fungal biomarkers therefore indicates that EMF hyphal networks were strong sinks for recent photosynthates. This is in line with previous studies that have highlighted the importance of an active photosynthate supply to the maintenance of EMF mycelial networks (Ellstrom et al., 2015; Hogberg et al., 2001).

4.3. C turnover in soil respiration

^{13}C in soil respiration was elevated above natural abundance just 4 h after labelling. As the canopy and soil were not physically separated it was not possible to determine whether this represented the time lag from plant assimilation, the abiotic return of ^{13}C, or a combination of the two. It has been shown in a forest stand that when soil is exposed to an atmosphere highly enriched in ^{13}CO$_2$, physical diffusion of ^{13}CO$_2$ from soil pores can be significant for up to 48 h after labelling (Subke et al., 2009). However soils under both miscanthus and SRC willow plantations were compacted agricultural soils with relatively high bulk density (miscanthus - 1.38 ± 0.05 (SE), SRC willow − 1.36 ± 0.04 g cm^{-3} (SE) for the top 0−15 cm) thus, compared to a typical forest soil, presumably less soil pore space was available for the abiotic return of ^{13}CO$_2$. The % of air filled pore space was lower under miscanthus (7.78 ± 3.55 1 SE) relative to SRC Willow (23.70 ± 3.35 1 SE) during the 48 h after ^{13}C labelling which may explain some of the early observed difference in ^{13}C flux between the two crops. However, the magnitude of the difference up to 7 days after labelling strongly suggests a biotic response. The time lag from labelling to peak enrichment in soil respiration was 48 h for both SRC willow and miscanthus. This is comparable to values observed on poplars, also members of the *Salicaceae* family and again suggests rapid belowground C transfer and turnover in both crops (Horwath et al., 1994; Mikan et al., 2000).

In agreement with our prediction that SRC willow would allocate more C belowground, ^{13}C flux was 5 times higher under SRC willow relative to miscanthus during the first 7 days after labelling. This may be linked to EMF dominance under SRC willow as Pumpanen et al. (2008) showed that 9−26% of all recently assimilated plant C is respired from EMF infected roots and increases in the rate of soil respiration have been observed in response to EMF infection (Leake et al., 2001). Root respiration accounts for on average, 40−50% of total ecosystem respiration (Bond-Lamberty et al., 2004; Hanson et al., 2000) and therefore higher rates of soil respiration in SRC willow compared to miscanthus may also be related to differences in the structure and depth distribution of below ground biomass. Miscanthus have rhizomes which have been shown to account for 66% of belowground biomass in an 11 year old miscanthus field (Clifton-Brown et al., 2007). These may create localized hotspots of respiration which may not have been captured by the static chamber method utilised. It has also been shown that herbaceous bioenergy crops including miscanthus have higher fine root biomass below the plough depth (0−30 cm) than woody species such as SRC willow (Chimento and Amaducci, 2015). Therefore, C allocated belowground under miscanthus may be less exposed to mineralization as microbial biomass, turnover time and C uptake rates generally decrease with depth (Spohn et al., 2016).

4.4. Potential implications for carbon cycling and storage

In this study we found that SRC willow plots had greater F:B ratios and allocated a greater proportion of recent assimilates into fungal (saprotrophic and ectomycorrhizal) biomass relative to miscanthus. The F:B ratio has been widely used as a simple soil microbial index and it has been hypothesised that increases in fungal dominance may lead to alteration of ecosystem processes such as increased C sequestration (Jastrow et al., 2007; Strickland and Rousk, 2010). However, most studies to date have been comparative and direct evidence to support this hypothesis is scarce (Bailey et al., 2002; Malik et al., 2016). This hypothesised relationship has commonly been attributed to a higher growth yield efficiency of fungi relative to bacteria, although studies have shown significant overlap between the two (Dijkstra et al., 2011; Six et al., 2006; Thiet et al., 2006). Fungi are also assumed to have longer rates of biomass turnover and thus residence time of C, (Rousk and Baath, 2007a, 2007b), and more recalcitrant necromass relative to bacteria (Guggenberger et al., 1999; Li et al., 2015). Fungal activity may also lead to increased soil aggregation through physical processes such as particle entanglement with hyphae and direct physical protection (e.g. hyphal growth into coarse woody debris/soil aggregates) (Guggenberger et al., 1999; Six et al., 2006). Our findings do not support a positive relationship between fungal dominance and soil C sequestration as, despite greater allocation of C to fungal PLFA in SRC Willow relative to miscanthus, no differences were detected in ^{13}C enrichment of bulk soil (supplementary Information − S2). Moreover, more ^{13}C was lost in soil respiration (Fig. 7). This supports recent work by Rousk and Frey (2015) which found a positive relationship between fungal dominance and ecosystem C losses in temperate forests. However, continuous ^{13}C labelling or the use of ^{14}C is a more sensitive technique better suited to studying the dynamics of larger C pools, due to the significant dilution of the ^{13}C pulse signal in large C pools such as SOM (Kuzyakov and Domanski, 2000; Studer et al., 2014; Trumbore, 2009).To further our mechanistic understanding of the links between microbial community composition, activity and soil C sequestration in perennial bioenergy systems, further study is required tracing assimilated C belowground into root exudates, microbial biomass, soil aggregates and fractions. However, our study does elucidate the important roles that soil fungi and bacteria play in the turnover of recently assimilated C under SRC willow and miscanthus respectively.

5. Conclusions

- Upper miscanthus leaves acted as strong C sinks with a higher CUE relative to SRC willow. This was likely due to differences in phenological stage rather than differences in their photosynthetic pathways.
- Labelled assimilates were transferred to roots and respired in under 4 h with similar transfer rates between the two crops. The ^{13}C flux in soil respiration was greater from SRC willow and may be due to increased belowground allocation to support EMF and a shallower root distribution relative to miscanthus.
- Distinct microbial PLFA profiles were present under adjacent SRC willow and miscanthus plots with a common previous management history. Microbial C cycling was fungus dominated in SRC willow soils whilst Gram positive and Gram negative bacteria were dominant in cycling labelled assimilates in miscanthus.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.07.026.

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