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Title: **Plant identity control on soil food web structure and C transfers under perennial bioenergy plantations**

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Abstract

Conversion from arable cropping systems to perennial bioenergy crops is increasing across Europe to meet market energy demands, but our understanding of how this land use change is affecting below-ground diversity and C allocation remains limited. Here, we assessed the impact of conversion from arable cropland to *Miscanthus* and Short Rotation Coppice (SRC) willow in UK bioenergy commercial plantations on earthworm community structure and abundance. At this same location we then conducted an in-situ $^{13}CO_2$ pulse-chase labelling experiment in the two bioenergy plantations to trace the fate of recently photosynthetically assimilated carbon into roots, bulk soil, soil microbial (PLFA) and earthworm communities. Results showed that land conversion from annual arable crops to both *Miscanthus* and SRC willow perennial bioenergy crops led to severe reductions of soil earthworm abundance and biomass. SRC willow had higher microbial biomass relative to *Miscanthus*, whereas *Miscanthus* provided a better habitat for a more functionally diverse earthworm community. Transfer of labile C compounds to soil pools was faster under *Miscanthus* supporting activity of bacterial grazers in the soil food chain, whereas a fungal-driven detrital decomposition processes dominated under SRC willow plantations. Taken together, these findings indicate that plant identity in land conversions have a strong influence not only on the abundance and structure of soil communities but also on which basal resources (root exudates, dead organic matter or microbial derived compounds) are preferentially consumed, and ultimately on the rates of mobilisation of these different C pools.

Keywords: *Miscanthus; SRC willow; ^{13}C pulse labelling; Earthworms; Bacteria; Fungi; Bioenergy crops*

1. Introduction

Bioenergy and biofuel production from temperate, perennial bioenergy crops has been proposed as one strategy to mitigate greenhouse gas emissions in the transport and energy generation sectors across Europe. Converting annually arable lands to perennial cropping systems can result in

substantial reductions of CO_2 and N_2O emissions, erosion and nutrient leaching, and in greater soil organic matter storage (e.g. Fazio and Monti, 2011; Schmer et al., 2014; McCalmont et al., 2017; Zhu et al., 2018). In addition, farmland biodiversity (both above- and below-ground) seems to benefit from increased spatial and structural heterogeneity provided by these agro-ecosystems with low mechanical and chemical input (e.g. Everaars et al., 2014; Bourke et al., 2014; Schrama et al., 2014; Haughton et al., 2016; Petrovan et al., 2017; McCalmont et al., 2017; Platen et al., 2017; Landis et al., 2018). However, the observed impacts may vary depending on the crop type, previous land-use and the years under cultivation (Schrama et al., 2014; Dauber et al., 2015, Petrovan et al., 2017). This is because ultimately vegetation drives the amount and quality of plant inputs to soils as well as exerting influence on soil abiotic conditions, which in turn shape below-ground heterotrophic communities (van der Putten et al., 2013; Barney, 2014; Oates et al., 2016).

In the UK, 129,000 hectares of agricultural land were used to grow bioenergy crops in 2017 (equivalent to just over 2% of total arable land), with 0.1-0.2% of this land dedicated to grow *Miscanthus* and short rotation coppice (SRC) willow for the heat and electricity energy markets (DEFRA, 2019). Unlike the conventional agricultural cultivations, these lignocellulosic crops are tall plants (3 m for *Miscanthus* and up to 8 m for SRC willow), which are planted in low density plantations and have deeper rooting systems extending up to 2.5 m depth, although the maximum root density concentrates around 0.36 and 1.2 m for SRC willow and *Miscanthus*, respectively (Finch et al., 2009). *Miscanthus* × *giganteus* (commonly known as "Giant or Elephant grass") is a warm season C4 grass native to Asia and the result of a naturally occurring triploid hybrid between the diploid *Miscanthus sinensis* and the tetraploid *Miscanthus sacchariflorus* (Stewart et al., 2009). Various willows species (*Salix* spp.) are used as biomass crops, with the majority of them being native to China (Weih, 2013). They are C3 plants like the majority of the plants in Europe and their higher quality litter (i.e. lower C:N ratio) decomposes within a year (Harris et al. 2017), whilst that of *Miscanthus* tends to accumulate on the soil surface (3–7 Mg dry matter ha^{-1} yr^{-1}; reviewed by Amougou et al., 2012). Although both crops can form mycorrhizal associations, they associate with different groups: *Miscanthus* associates

exclusively with arbuscular mycorrhizae (AM) whilst in SRC willow, although capable to host both arbuscular and ectomycorrhizae (ECM), usually ECM dominate (Kahle et al., 2005). This distinction not only refers to differences in mycorrhizal anatomy, but also to their enzymatic capabilities (broader in the case of ECM, allowing them to forage from labile and recalcitrant organic pools) and hence, controlling the amount of nutrients allocated in the roots and exuded to the surrounding soil (Churchland and Grayston, 2014). Therefore, conversions of arable crops to these perennial bioenergy crops involve not only changes to above-ground plant communities with different C metabolic pathways, but also to below-ground heterotrophic communities.

Soil fauna play a key role in decomposition processes and, among them, macrofauna (earthworms) are considered to be key "ecosystem engineers" because they create biogenic structures (burrows and casts) that represent important habitats for other soil organisms (Lavelle et al., 2016). According to their feeding and burrowing strategies, they can be classified into three different ecological groups (*sensu* Bouché, 1977): epigeic worms burrow and feed on soil upper layers, anecic worms that incorporate fresh organic residues deposited at the soil surface along the soil profile, and endogeic worms feeding on more mineralised organic matter in the deeper soil layers. They are very sensitive to agricultural practices and as result, their populations have been shown to be severely reduced by intensive management of soils (Tsiafouli et al., 2015; Briones and Schmidt, 2017). This has also led to their consideration as "biological indicators" of the status of agroecosystems (Perés et al., 2008; Van Eekeren et al., 2008; Postma-Blaauw et al., 2010). From this, it is expected that in these no till soils that receive low external inputs (in terms of mineral fertilisers and herbicides), earthworm activities will be enhanced. Indeed, several studies have reported increases in earthworm abundance and diversity under perennial bioenergy crops (Felten and Emmerling, 2011; McCalmont et al., 2017). However, planting species with low litter quality (such as *Miscanthus* spp.) could result in a higher competition for N between soil microorganisms and these soil invertebrates (Ernst et al., 2009) leading to individual biomass reductions (McCalmont et al., 2017), and ultimately slowing down decomposition processes (Ernst et al., 2009; Amougou et al., 2012).

Because plant identity plays a critical role in rhizosphere responses (Emery et al., 2017), and both biomass and biofuels can be derived from different energy crops with different functional traits (e.g. grasses vs. trees, C4 vs. C3 pathways), it is crucial to get a better understanding of the dynamics of C flow and storage below-ground under different types of crops. *In situ* isotopic tracer studies provide quantitative information on C transfers from plant tissues to the surrounding soil and thereby, how much C can be translocated by soil organisms. More specifically, field $^{13}CO_2$ pulse labelling experiments have allowed estimations of the residence times of photoassimilated carbon into rhizosphere biota (e.g. Ostle et al., 2007; Seeber et al., 2012; Crotty et al., 2014; Huang et al., 2015). Nevertheless, very few have been performed in bioenergy crops (Tavi et al., 2013; Chaudhary and Dick, 2016; Elias et al., 2017) and our study is the first one trying to link soil macrofauna and rhizosphere carbon flow in these systems.

In this study, we firstly assessed the impact of land conversion from arable to *Miscanthus* and SRC willow on earthworm communities, and then used a combination of natural abundance $\delta^{13}C$ and $\delta^{15}N$ measurements and $^{13}CO_2$ labelling to track assimilation of recently fixed photosynthates into rhizosphere organisms. We hypothesised that lower physical disturbance under no tilled soils would enhance earthworm abundance and functional diversity in agreement with previous studies (Briones and Schmidt, 2017). We also anticipated that, due to differences in root morphology and associated symbionts as well as in litter quality, earthworm and microbial (PLFA) community structures would differ between *Miscanthus* and SRC willow. The study took advantage of a parallel study performed at the same site (Elias et al., 2017) which found a higher below-ground C allocation under SRC willow (relative to *Miscanthus*) that was rapidly assimilated by fungi. We therefore predicted higher heterotrophic activities under this plantation and a rapid assimilation of labile C by soil biota through fungal grazing.

2. Materials and methods

2.1. Bioenergy crops

The research sites were two adjacent commercial bioenergy plantations of *Miscanthus giganteus* (11.56 ha) and short rotation coppice (SRC) willow (*Salix viminalis* L.; 9.43 ha) in Lincolnshire, UK. Meteorological data obtained from the nearest weather station (RAF Scampton, Lincoln; 53° 18′ 1″N, 0° 32′ 30″W) showed mean annual minimum and maximum temperatures of 5.9 °C and 13.1 °C respectively (1971-2000) and a mean annual rainfall of 605 mm (1963 to 2004; Drewer et al., 2012).

Both crops were planted in 2006 and 2000, respectively, on arable land that was previously managed on a rotation of 1-year oilseed rape followed by 3 years winter wheat. Directly prior to conversion, both bioenergy crop fields had 3 years of wheat cultivation (Drewer et al., 2012). Although the two bioenergy crops did not receive any N fertilizer in the duration of the project (or during the establishment phase), the annual crop received three to four applications of N-fertilizer every spring (equalling to 186, 189, and 209 kg N ha^{-1} yr^{-1} in 2009, 2010 and 2011, respectively). Despite these N additions, the soil analyses revealed no significant differences in the total soil N content between the three fields (Table 1).

The soil of the three fields was a silty clay loam (3% sand, 60% silt and 37% clay, on average; Table 1) with a low soil water content (ranging from 33 to 37%, on average), being slightly higher under the two bioenergy plantations than in the adjacent annual arable field (Table 1). Total C and N were also very low in all three fields (1.64 %C and 0.27 %N in *Miscanthus*, 1.56 %C and 0.24 %N in SRC willow and 1.80 %C and 0.29 %N in the arable soil; Table 1). Bulk density was 1.48 ± 0.11 g cm^{-3} in *Miscanthus*, 1.45 ± 0.11 g cm^{-3} in SRC willow and 1.25 ± 0.09 g cm^{-3} in the arable system (Table 1). Finally, the soil pH values indicated that the three soils were slightly acidic, with the lowest values being measured under *Miscanthus* than in the other two systems (Table 1).

140 *2.2. Pre–^{13}C pulse measurements*

141 Earthworms were collected from each plantation (four quadrats of 50 cm x 50 cm x 10 cm deep)
142 by hand-sorting. Additional samples were also taken from the adjacent arable soil to test the effects
143 of changes in land use on earthworm abundance and biomass. All specimens collected were identified
144 to species level under a microscope, counted and weighed (fresh biomass).

145 Leaf litter samples were also collected at both *Miscanthus* and SRC willow experimental plots,
146 transferred to labelled bags and immediately stored in a cool box. Samples were subsequently frozen
147 (-20 °C), before being oven dried at 60 °C and cryo – milled (SPEX SamplePrep, Freezer/Mill 6770) to a
148 fine powder for isotopic analysis.

149 Three soil cores (2.5 cm diameter gouge auger; Eijkelkamp Agrisearch Equipment, Giesbeek,
150 Netherlands) were taken from each experimental plot, sectioned into two depths (0-10 cm and 10-20
151 cm) and frozen at -20 °C within 2 h of collection. These were subsequently freeze-dried (Christ alpha
152 1-4 LD Plus) and then sieved to 2 mm. Stones were removed while root and rhizome samples were
153 transferred to glass vials. The remaining soil was ball milled (Fritsch Planetary Mill Pulviresette 5) to a
154 fine powder. Picked roots were washed, oven dried at 60 °C and cryo – milled (SPEX SamplePrep,
155 Freezer/Mill 6770) to a fine powder. Bulked subsamples of the 0-10 and 10-20 cm freeze dried ground
156 soil were used for PLFA analyses to determine the microbial community structure under each system.

157 Finally, natural abundance $\delta^{13}C$ and $\delta^{15}N$ signatures of plant litter, roots/rhizomes, soils, and
158 earthworms collected from each bioenergy plantation were also obtained.

159

160 *2.3. $^{13}CO_2$ pulse labelling experiment*

161 Within each plantation, four rectangular pulse chambers (6 m long x 2.5 m wide x 3 m high) were
162 erected over the entire experimental plot (for full description of the experimental set-up see Elias et
163 al., 2017). Briefly, a chamber volume of 45 m^3 allowed for the inclusion of two double rows of willow

trees 1.5 m apart, whilst *Miscanthus giganteus* was randomly distributed in the 15 m^2 area. Clear polythene sheeting, allowing 90% of photosynthetically active radiation to penetrate, was placed over the frame and sealed at ground level. One 6.5 Kw water cooled, split air conditioning unit (Andrew Sykes, UK) and 2 tripod mounted fans were installed within the chambers to counter ambient temperature increases during the ^{13}C pulse and to ensure homogeneous distribution of $^{13}CO_2$. Pulse labelling commenced August 23rd, 2012 at ca. 08:20 am by introducing 17 L of 99 atom% pure $^{13}CO_2$ (CK Gases, UK) to each chamber 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 h in order to counter an above ambient CO_2 concentration increase. During the pulsing period (4.5 h), $\delta^{13}C$ 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; Elias et al., 2017).

2.4. Post – $^{13}CO_2$ pulse sampling

Previous studies have shown that the incorporation of dietary C into earthworm body tissues is typically slower than in microbes (>20 days; Ostle et al., 2007; Dungait et al., 2008), and for this reason, our sampling dates were chosen to maximise the incorporation of the ^{13}C label into the soil biota (either via aboveground litter or belowground roots/rhizomes). Since senescence of willow leaves occurred between 42 and 76 days (Elias et al., 2017), root/rhizomes and soil samples (including for PLFA) as well as earthworms were sampled 21, 28, 42 and 76 days after labelling. The last sampling coincided with the end of the senescence period of willow leaves (October 2012).

On each sampling occasion, four soil cores were taken using the same methodology described above. Similarly, the soil samples collected were frozen at -20 °C, subsequently freeze - dried and finally sieved (< 2mm) to obtain the root/rhizome and soil material for further isotopic analyses. Earthworms were hand-sorted in the field from four quadrats (25 x 25 x 10 cm) and identified to

189 species level under a microscope. Thereafter, the gut of every specimen was removed and the tissues
190 washed with deionised water to remove any soil particles. Earthworm tissue samples were then frozen
191 at -20 °C for at least 24 h prior to freeze-drying and then weighed using a Sartorius (UK) microbalance
192 to determine their dry weight.

193 *2.5. Bulk isotopic analyses of soil, litter, root/rhizomes, and earthworm samples*

194 $\delta^{13}C$ and $\delta^{15}N$ measurements of soils, earthworm tissues and plant materials (leaf litter and
195 roots/rhizomes) were performed by weighing subsamples of each of these materials into tin capsules
196 followed by combustion using a CarloErba elemental analyser. Resultant CO_2 and N_2 was analysed for
197 $\delta^{13}C$ and $\delta^{15}N$, respectively, using a Dennis Leigh technology IRMS at the NERC Life Sciences Mass
198 Spectrometry Facility, CEH Lancaster. Beet sucrose (-26.46‰) was used as an instrument standard for
199 $\delta^{13}C$ and n-Carbobenzyloyx-L-aspartic acid (-5.55‰) for $\delta^{15}N$ were used as an instrument standards.
200 The standard deviation for duplicate and QC samples was not more than 0.47‰ for $\delta^{13}C$ and 1.07 for
201 %C, and not more than 0.69‰ for $\delta^{15}N$ and 0.05 for %N, resulting in an analytical precision of 0.22‰
202 for $\delta^{13}C$ and 0.53‰ for $\delta^{15}N$.

203 Isotopic values are expressed in delta notation:

204 $$\delta^{n}X = (R_{sample} / R_{standard} - 1) \times 1000\ (‰)$$

205 where X = C (carbon) or N (nitrogen) and R = $^{13}C/^{12}C$ for C and $^{15}N/^{14}N$ for N.

206 All $\delta^{13}C$ results are expressed relative to the international standard Pee Dee Belemnite and all $\delta^{15}N$
207 results are expressed relative to the international standard of atmospheric air.

208

209 *2.6. PLFA extraction and ^{13}C - PLFA analyses*

210 PLFA biomarkers were extracted as part of the total lipid extract of freeze-dried soil samples using
211 a modified Bligh-Dyer extraction (White et al., 1979). For full description of the PLFA extraction

procedure and full QA/QC see Elias et al. (2017). 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 and 7,8 cy-C19:0 were used as indicators of Gram negative bacteria (Rinnan and Baath, 2009). The fatty acids C18:2ω6,9c and 18:1ω9c were taken as indicators of fungi (Kaiser et al., 2010). Total microbial biomass was taken as the sum of all identified PLFA's (n = 17; C16:0, C16:1ω5c, 10Me-C16:0, C17:0, 10Me-C17:0 and C18:0 plus those 11 listed above).

Each of the individual PLFA's identified above 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 more details of the instrumental set up, see Elias et al. (2017).

2.7. Mass balance calculations and statistics

All enriched results were converted to atom% excess (please see supplementary material for the detailed descriptions of these calculations). To determine the preferential C substrate utilization (i.e. soil or root-derived) by microorganisms and earthworms, the following equation was used to calculate the fractional input (F) of C from the ^{13}C pulse source into *Miscanthus* (M) and willow (W) C pools (Ostle et al., 2007; Crotty et al., 2011; see also supplementary material):

$$F_Y = (^{13}C_Y - {}^{13}C_{NAY})/(^{13}C_{ZY} - {}^{13}C_{NAZY})$$

where Y = crop: M (*Miscanthus*) or W (willow), Z = sample (soil or root sources), $^{13}C_Y$ is the atom% excess value of the C assimilated into the microbe/earthworm tissues under each crop after labelling and $^{13}C_{NAY}$ is the atom% excess value of C assimilated in the reference population (natural abundance, pre-pulse); $^{13}C_{ZY}$ is the atom% excess value of the soil and root/rhizome under *M. giganteus* or willow after labelling and $^{13}C_{NAZY}$ is the natural abundance atom% excess value of the soil and root/rhizome samples taken before pulse-labelling (baseline, pre-pulse) under each vegetation type. This calculation assumes equal isotopic fractionation by humification of C_3 plants and C_4 plants (Schneckenberger and Kuzyakov, 2007).

The final quantities of pulse derived ^{13}C incorporation into microbial PLFAs and earthworms were finally expressed as pulse-derived ^{13}C (mg C per mg total dry matter) using the following equation (Ostle et al., 2007):

$$\text{pulse - mg C per mg total dry matter} = F_Y \text{ X biomass-C}$$

where biomass-C (mg C per mg total dry matter) is the amount of carbon within the biomass of a sample (dry weight).

Analyses of Variance (ANOVA) was used to evaluate differences in microbial communities and earthworm groups across land use type (i.e. arable, *Miscanthus* and SRC willow). Repeated measures ANOVA was used to determine the influence of time and type of bioenergy crop (*Miscanthus* and SRC willow) on pulse- derived ^{13}C content (mg C per mg total dry matter) of PLFAs and earthworm tissue samples. Normality and homogeneity of variances were checked using the Shapiro-Wilk test and the Levene's test, respectively, and data were log transformed (log10 (x+1)) when necessary.

All statistical analyses were performed using SAS System Release 9.3 (SAS Institute Inc., Cary, NC). Differences for all analyses were considered significant when $P<0.05$. The data are presented as means of four replicates ± S.E. unless stated otherwise.

3. Results

3.1. Effects of land use change on earthworm and microbial communities

Both SRC willow and *Miscanthus* plantations had significantly lower earthworm abundance (81 and 87% reduction respectively; $p = 0.0012$) and biomass (64 and 75%, respectively; $p = 0.0013$) relative to the adjacent arable field (Fig. 1). This land use change also resulted in the alteration of the functional structure of their communities ($p < 0.0001$), with significant decreases in the abundance of epigeic and endogeic worms under both SRC willow and *Miscanthus* and the total absence of anecics under SRC willow (Fig. 2).

Miscanthus soils not only contained more earthworms but also a higher species richness relative to SRC willow (Figs. 1 and 3a). In contrast, total PLFA concentrations were higher under SRC willow relative to *Miscanthus* ($p_{Fungi} = 0.0003$, $p_{Bacteria} = 0.0253$, $p_{GP} = 0.0459$, $p_{GN} = 0.0169$, $p_{Unspecific} = 0.0019$). Fungal PLFA's concentrations in particular were almost double those measured in *Miscanthus* (Fig. 3b). However, bacteria was the dominant group in both plantations (54 and 56% of the total PLFA concentrations: Fig. 3b).

3.2. Natural abundance isotopic composition of below-ground communities under Miscanthus and SRC willow

Natural abundance $\delta^{13}C$ values of *Miscanthus* and SRC willow below-ground tissues were a reflection of the two different photosynthetic pathways (C3 and C4 metabolism), with *Miscanthus* rhizomes showing a ^{13}C enrichment of ca. 15 delta units compared to the C3 willow roots ($p < 0.0001$; Fig. 4). In contrast, the soils supporting these two plantations were very similar in their isotopic signatures (Fig. 4), suggesting that C4 inputs from *Miscanthus* are not yet measurable in the bulk C3 dominated soil. These results were consistent across the two soil layers sampled here ($p_{depth} > 0.05$). Therefore, samples from different depths were pooled in further statistical analyses.

283 Surprisingly, the identity of plant species had very little effect on the natural abundance values of
284 microbial communities, with the exception of fungi (Fig. 5). Thus, Fungal PLFA were more enriched
285 under *Miscanthus* compared to SRC willow, suggesting a closer association with the rhizomatous
286 system of this perennial grass (Fig. 5b). By contrary, the natural abundance values of the other three
287 microbial groups were, on average, similar to those measured in the bulk soils under the two crops
288 (Fig. 5). The only exception to this were the Gram negative bacteria in the willow plantations, by being
289 significantly ^{13}C depleted compared to the bulk soil, although showing a higher isotopic enrichment
290 than the willow roots (Fig. 5a).

291 Earthworm populations showed a distinct preference for the different substrates available (Fig. 6).
292 All individuals collected under *Miscanthus* were enriched in $\delta^{13}C$ (> -15‰) compared to those
293 measured under SRC willow (<-24‰) (Fig. 6). Furthermore, $\delta^{15}N$ isotopic ratios clearly differentiated
294 the different earthworm ecological groupings, with the epigeic worms feeding preferentially on fresh
295 plant inputs (litter), and endogeics assimilating the most mineralised soil organic matter at both
296 systems (Fig. 6). Moreover, the isotopic values of the anecic species collected under this C4 crop were
297 closer to the rhizome isotopic values than to the litter, suggesting that they were feeding on root
298 exudates or on the microorganisms living around the *Miscanthus* rhizome system. Interestingly, $\delta^{15}N$
299 values of the endogeic worms, although relatively close to those of the soils, were higher under
300 *Miscanthus* than under SRC willow indicating greater assimilation of more humified substrates (Fig.
301 6).
302
303 *3.3. Below-ground translocation of recently photosynthesised ^{13}C carbon*
304 Translocation of photosynthetically assimilated ^{13}C into plant roots was detected over the whole
305 studied period, but the difference in ^{13}C enrichment between the two crops only became significant
306 76 days after labelling (Repeated measures ANOVA for the time factor $p = 0.0092$ and for the
307 interaction crop*time: $p = 0.0367$), with SRC willow roots increasing their isotopic enrichment by three
308 fold, when compared to *Miscanthus* rhizomes (average enrichments of 0.023613± 0.005384 (S.E.) and

309 0.0070316±0.0014288 ^{13}C %atom excess in SRC willow and *Miscanthus*, respectively). For bulk soils,
310 significant differences between crops occurred slightly earlier, on day 42 (Repeated measures ANOVA
311 for the time factor p = 0.2049 and for the interaction crop*time: p = 0.0423); however, on this
312 occasion, soils under *Miscanthus* showed a higher enrichment than those under willow (average
313 enrichments of 0.002192984±0.000511258 (S.E.) and 0.0001418±0.000334361 ^{13}C %atom excess in
314 *Miscanthus* and SRC willow soils, respectively).

315 Peak enrichment of pulse derived ^{13}C from both roots and soils into microbial PLFA was only
316 significant 42 days after labelling. Only two groups, Gram (-) bacteria and Unspecific PLFA collected
317 under SRC willow showed significant differences in ^{13}C recovery compared with the same microbial
318 groups under *Miscanthus* on that date (Repeated measures ANOVA for the time factor: p_{GN} = 0.0007,
319 $p_{Unspecific}$ = 0.0222 for root-derived ^{13}C and p_{GN} = 0.0022, $p_{Unspecific}$ = 0.0308 for soil-derived ^{13}C).
320 However, the enrichment was far more pronounced when the incorporated photosynthate carbon
321 was derived from soil rather than from roots (Fig. 7).

322 Earthworms followed a similar pattern as microbial communities since the populations under
323 willow exhibited a significantly higher ^{13}C enrichment on day 42, when the highest amounts of tracer
324 were also observed in the soils. Although a significant ^{13}C enrichment were also observed in the
325 earthworm tissues after 76 days that could be attributed to a greater assimilation of labelled C
326 substrates derived from the *Miscanthus* rhizomes, this incorporation was also less marked than that
327 from SRC willow soils (Fig. 8).

328

329 **4. Discussion**

330 *4.1. Land use effects on rhizosphere biota*

331 In this study, we found that land conversion from annual arable crops to perennial bioenergy
332 plantations of *Miscanthus* and SRC willow caused significant reductions in the abundance and biomass

of earthworm communities. This finding contrasts with previous results indicating that earthworm densities and diversity are higher under these perennial crops than in classical arable soils, as result of decreasing land use intensity (Makeschin, 1994; Felten and Emmerling, 2011; Lagerlöf et al., 2011; Stauffer et al., 2014). At the two bioenergy crops, the only disturbance was caused by the annual harvests for *Miscanthus* and three yearly harvest for SRC willow. However, the least disturbed SRC willow contained the lowest earthworm abundances and number of species. Clearly, other factors besides land management have a more predominant role in structuring earthworm communities at the investigated site.

The most dominant ecological group in the three agriculture systems were endogeic earthworms. This dominance of endogeic worms in agricultural soils has been consistently reported in the literature (reviewed by Briones and Schmidt, 2017) and it is because these species concentrate their activities in mineral soil layers where they are less exposed to disturbances, compared to the more exposed epigeic and anecic worms that feed on the soil surface. These ecological groups are very sensitive to agricultural management and drastic declines have been observed in both tilled and no tilled soils (e.g. Briones and Schmidt, 2017; Lago et al., 2019). However, the significant reduction of these surface feeders observed at the two bioenergy plantations cannot be solely due to agricultural practices (i.e. fertilisation, weeds control, etc.). The lignin content of the perennial biomass crop litter is high: 27.6% of ash-free dry mass in the case of SRC willow (Slapokas and Granhall, 1991) and 28% in the cell walls of *M. gigantheus* (Lygin et al., 2011). In addition, *Miscanthus* litter is not a nutritious food source due its high C:N ratios (83-100; Amougou et al., 2012). These non-palatable substances repel phytophagous insects and can also hamper microbial and earthworm attack (Slapokas and Granhall, 1991). However, it has been suggested that the extensive litter cover at ground level under *Miscanthus* can provide earthworms with a greater soil surface moisture retention and protection from predation (McCalmont et al., 2017). This could explain why higher earthworm numbers and biomass (and in particular of those species concentrating their activities at the soil surface) were collected under this

358 grass species than under SRC willow, although the differences between the two crops were not
359 significant.

360 In contrast to earthworms, the soils under SRC willow contained more microorganisms, in
361 particular higher concentrations of total fungi and bacteria, which is consistent with the idea that
362 hyphal exudations from ECM trees support a more diverse and active bacterial populations (Tedersoo
363 et al., 2009, Bomberg et al., 2011, Churchland and Grayston, 2014; Taylor et al., 2016). These findings
364 have been used as a solid evidence for stronger rhizosphere effects under ECM stands (Yin et al., 2014)
365 due to their broad enzymatic capabilities for mining nutrients from soil organic matter (Cheeke et al.,
366 2017).

367

368 *4.2. Effects of bioenergy plantations on C transfers through detrital food webs*

369 In the parallel study performed at the same site (Elias et al., 2017) no significant differences in the
370 transfer rates of labelled assimilates from leaves and stems to roots were observed between these
371 two perennial crops. However, in this study we observed significant differences in the below-ground
372 C allocation dynamics in the rhizosphere, with enrichment peaks in roots/rhizomes and bulk soils
373 occurring at different times at each crop. The shorter time lag in the case of *Miscanthus* soils could be
374 related to a greater exudation of more labile compounds in AM soils than in ECM soils (Finzi and
375 Canham, 1998; Finzi et al., 1998). Consequently, ECM plants like SRC willow tend to invest more in
376 fungal biomass than AM stands (Cheeke et al., 2017) that are strong sinks for recent photosynthates
377 (Elias et al., 2017). Furthermore, since ECM associations have also greater capabilities than AM and
378 soil bacteria to degrade recalcitrant substrates, they can survive in nutrient poor environments
379 (Wardle et al., 2004).

380 In addition, it is thought that ECM stands tend to reduce decomposition rates by producing
381 recalcitrant compounds which often bind N and hence, depriving soil decomposers from this essential

382 nutrient (the so-called “slow decay hypothesis”; Averill et al., 2014; Averill, 2016; but also see Craigg
383 et al., 2018). Earthworm populations were less abundant and diverse under SRC willow suggesting
384 that nutrient availability might be limiting the activities of these invertebrates under this crop. Both
385 earthworms and microbes (PLFA) assimilated more ^{13}C derived from bulk soil relative to root pools,
386 suggesting that the food webs in these agricultural soils are detritus-based, relying on humified soil
387 organic matter than on recent root exudates. The higher dominance of endogeic species in the
388 earthworm communities, which are mainly soil feeders (as reflected in their higher ^{15}N isotopic
389 signatures), supports this conclusion. Indeed, previous work has shown that in the case of micro-
390 foodwebs, the turnover of organic resources from the previous crop cycle can be sufficient to support
391 their energy demands for at least two growing seasons (Glavatska et al., 2017).

392 Unlike the fungal energy channel, bacteria are more dependent on recent photosynthates (Garcia-
393 Pausas and Paterson, 2011) and represent the “fast cycle” (Moore et al., 2003). In support of this, the
394 parallel study covering a much longer period (Elias et al., 2017) showed that the ^{13}C enrichment in
395 bacterial PLFA was greater under *Miscanthus* relative to SRC willow. Since bacteria are considered to
396 be an important food source fuelling energy to soil invertebrates (Crotty et al., 2011; Briones et al.,
397 2014), this could explain the more abundant and functionally diverse earthworm populations
398 observed under *Miscanthus*. On the other hand, the strong variation in earthworm $\delta^{15}N$ observed
399 between the two plantations indicates the existence of some spatial variation in earthworm feeding
400 strategies, with endogeic worms collected under *Miscanthus* feeding on more degraded organic
401 matter than under SRC willow. This could indicate that these species are rather generalist consumers
402 able to feed on different sources depending on availability or environmental conditions. By being less
403 selective, they can also better adapt to land conversion changes (Crotty et al., 2014). In contrast to
404 primary decomposers (feeding directly on plant litter/detritus with adhering fungi and bacteria), such
405 as the epigeic worms, secondary decomposers feed on microbes and microbial compounds (Scheu
406 and Falca, 2000; Schmidt et al., 2004; Chahathaghi et al., 2005). Furthermore, since bioturbation
407 activities by earthworms have shown to trigger the change in microbial communities from fungal to

408 bacterial dominance (Frouz et al., 2013), greater soil mixing due to the presence of deep burrowers
409 (anecic worms) might have enhanced the bacterial channel under *Miscanthus*. Therefore, our dual ^{13}C,
410 ^{15}N analyses of earthworm communities confirmed not only previous observations indicating that non-
411 predatory invertebrates tend to form a continuum from primary to secondary decomposers, but also
412 that a land use change to SRC willow or *Miscanthus* could result in a shift to either more detrital or
413 more microbial diets, respectively (Chahathaghi et al., 2005).

414 Taken together these findings indicate that plant identity plays a key role in soil web structure and
415 function by providing a greater (*Miscanthus*) or lower (SRC willow) number of trophic niches. This
416 resulted in a fungal driven food chain under SRC willow and a bacterially derived soil fauna feeding
417 channel under *Miscanthus*. This is in agreement with previous studies showing that plant type can act
418 as a top-down driver of soil food web functioning (Crotty et al., 2014; Elias et al., 2017), and that the
419 continuous supply of quality food throughout the year could have a more influential role in shaping
420 earthworm communities than absence of ploughing alone (e.g. Schmidt et al., 2003: Roarty et al.,
421 2017). Therefore, not only can land conversions and agricultural practices have a strong influence in
422 the abundance and structure of soil communities (Tsiafouli et al., 2015; Briones and Schmidt, 2017),
423 the crop species planted could stimulate the consumption of different basal resources (root exudates,
424 dead organic matter or microbially derived compounds). This together with "feeding flexibility"
425 exhibited by soil organisms (Briones et al., 2010) that also allows them to rely on "older" organic inputs
426 from previous crop cycles (Schrama et al., 2014; Glavatska et al., 2017) determines their resilience to
427 habitat and management changes.

428

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436

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661 **Table 1** Summary of soil properties of the different crops (SD = standard deviation)

	pH	SD		Bulk Density (g/cm^3)	SD		Total N (%)	SD		Total C (%)	SD		Clay (%)	SD		Silt (%)	SD		Sand (%)	SD		Soil moisture (%)	SD	
Arable	6.68	0.15	**a**	1.25	0.09	**a**	0.29	0.04	**a**	1.80	0.28	**a**	40.03	4.97	**a**	57.46	4.28	**a**	2.51	0.91	**a**	33.18	1.89	**a**
Miscanthus	5.97	0.58	**b**	1.48	0.11	**b**	0.27	0.03	**a**	1.64	0.36	**a**	34.44	1.88	**b**	62.61	1.98	**b**	2.95	1.03	**a**	36.83	1.73	**b**
SRC Willow	6.75	0.14	**a**	1.45	0.11	**b**	0.24	0.02	**a**	1.56	0.23	**a**	35.51	0.72	**ab**	60.85	1.06	**ab**	3.63	0.82	**a**	35.98	1.99	**b**

662

663 **Figure legends**

664 **Figure 1.** Earthworm density (a) and biomass (b) recorded in soils (0-10 cm) under the different land
665 uses. Error bars are S.E. and different letters indicate significant differences between cropping
666 systems.

667 **Figure 2. E**arthworm community structure (ecological groupings) recorded in soils (0-20 cm) under the
668 different land uses: Error bars are S.E. and different letters indicate significant differences between
669 cropping systems per ecological grouping.

670 **Figure 3.** Relative earthworm species composition (a) and microbial community structure (b)
671 determined from % of earthworm species abundances and PLFA assigned to functional groups relative
672 to total values in *Miscanthus* and SRC willow soils (pre-labelling). Earthworm species: *Aporrectodea*
673 *caliginosa* (Ac), *Aporrectodea rosea* (Ar), *Aporrectodea longa* (Al), *Allolobophora chlorotica* (Ah),
674 *Lumbricus rubellus* (Lr), *Lumbricus castaneus* (Lc), *Lumbricus* spp. (Lsp). Microbial groups: Gram
675 positive (G+) - (C15:0i, C15:0a, C16:0i C17:0i and C17:0a), Gram Negative (G-) - (16:1u7c, 7,8 cyclic
676 C17:0, C18:1u7c and 7,8 cy-C19:0), Fungi - (C18:2u6,9c and C18:1u9c), Unspecified (nonspecific) -
677 (C13:0, C14:0, C14:1u5c, C15:0, C15:1u5c, C16:0, 10Me-C16:0, C16:1u7t, C16:1u9c, C16:1u5c, C17:0,
678 10Me-C17:0, C18:0i, C17:1u7c, C18:0a, C18:0, 10Me-C18:0, C18:1u7t, C18:1u12c, C18:1u5c, C18:2u6t,
679 9,10-cy-C19:0, C19:1u12c, C20:0, C18:3u6c, C20:1u9c, C18:3u3c, C20:2u6c, C22:0, C20:3u6c,
680 C20:4u6c, C20:5u3c, C24:0).

681 **Figure 4.** Natural abundance isotopic signatures of belowground vegetation and bulk soil sampled at
682 0-10 and 10-20 cm in SRC willow and *Miscanthus*. Error bars are S.E. and different letters indicate
683 significant differences between plant and soil samples per soil layer.

684 **Figure 5.** Natural abundance C isotopic signatures of PLFA microbial groupings, belowground
685 vegetation and bulk soil sampled between 0 and 20 cm depth in SRC willow (a) and *Miscanthus* (b).

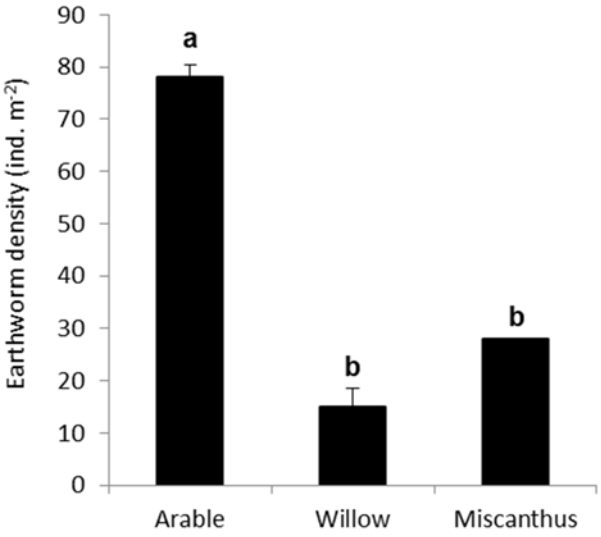
686 Box plot chart showing the median and quartiles (25th and 75th). Different letters indicate significant
687 differences between samples.

688 **Figure 6.** Natural abundance isotopic signatures (^{13}C and ^{15}N) of earthworm ecological groupings
689 (epigeics – EPI, anecics – ANE, endogeics – END) sampled between 0 and 10 cm depth in SRC willow
690 (W, white symbols) and *Miscanthus* (M, black symbols) together with the potential food sources (soil-
691 S, roots-R and litter-L). Error bars are S.E.

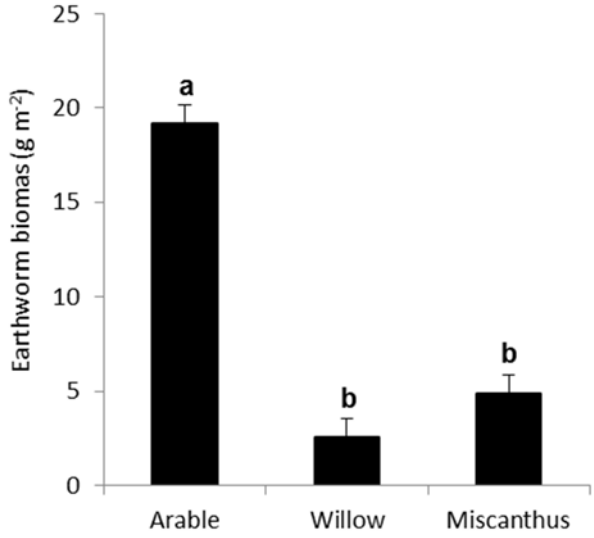
692 **Figure 7.** Root (a) and soil (b) pulse derived ^{13}C incorporation into Gram negative and Unspecific PLFA
693 collected under SRC willow and *Miscanthus* on day 42 after labelling. Box plot chart showing the
694 median and quartiles (25th and 75th).

695 **Figure 8.** Root (a) and soil (b) pulse derived ^{13}C incorporation into earthworms collected under SRC
696 willow and *Miscanthus* during the 76 days sampling period. Error bars are S.E. and the asterisk
697 indicates significant differences between cropping systems.

Figure 1


Earthworm density (ind. m-2)
90
80
70
60
50
40
30
20
10
0
a
b
b
Arable
Willow
Miscanthus

a


Earthworm biomas (g m-2)
25
20
15
10
5
0
a
b
b
Arable
Willow
Miscanthus

b

Figure 2

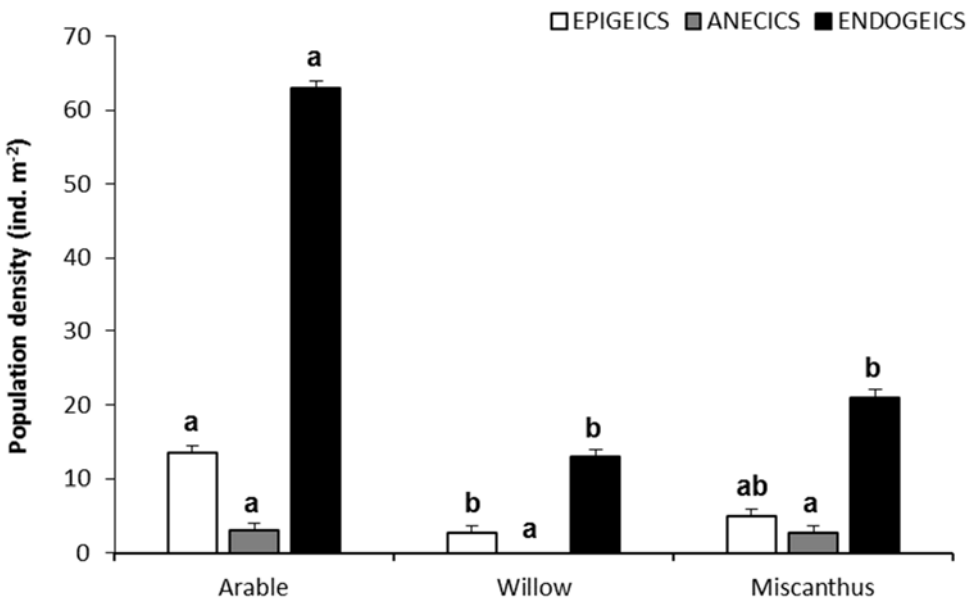
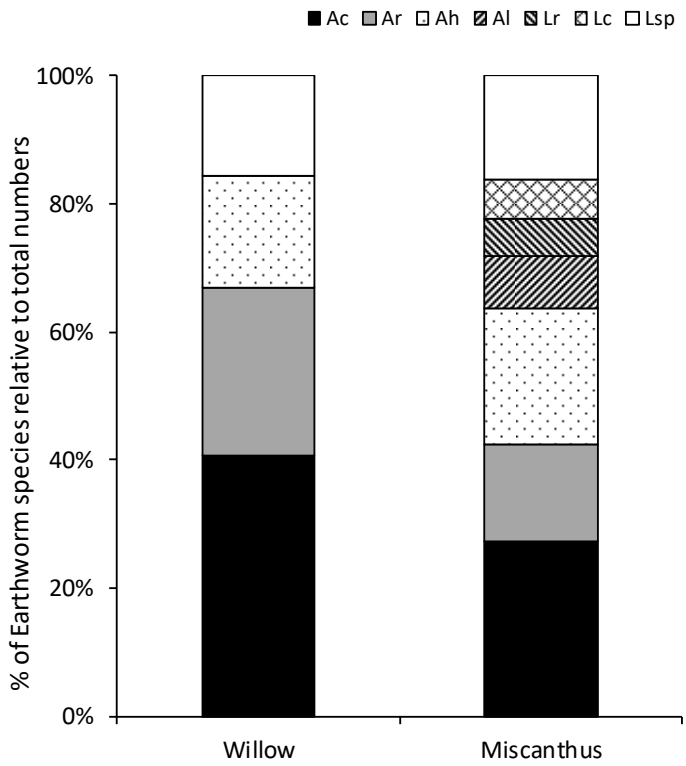
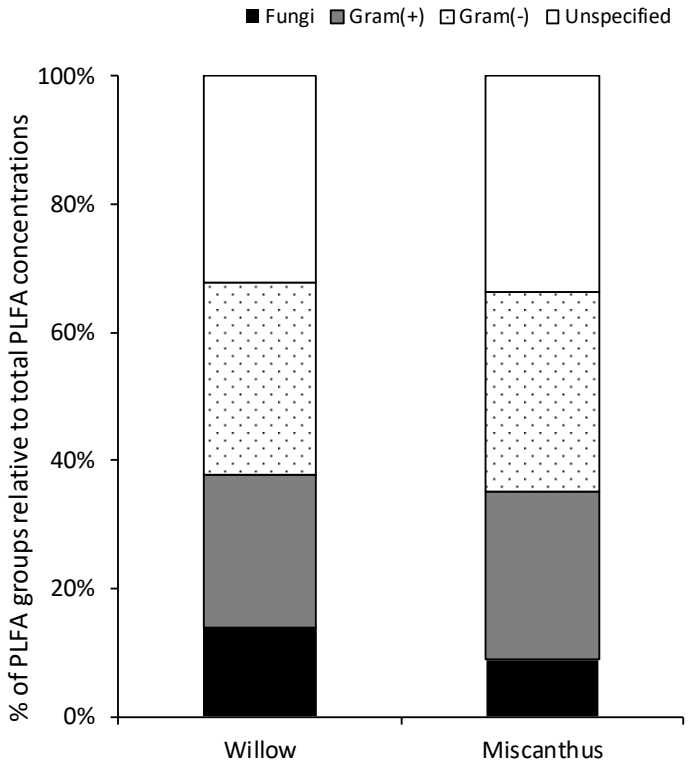

EPIGEICS
ANECICS
ENDOGEICS
Population density (ind. m-2)
70
60
50
40
30
20
10
0
a
a
a
b
a
b
ab
a
b
Arable
Willow
Miscanthus

Figure 3

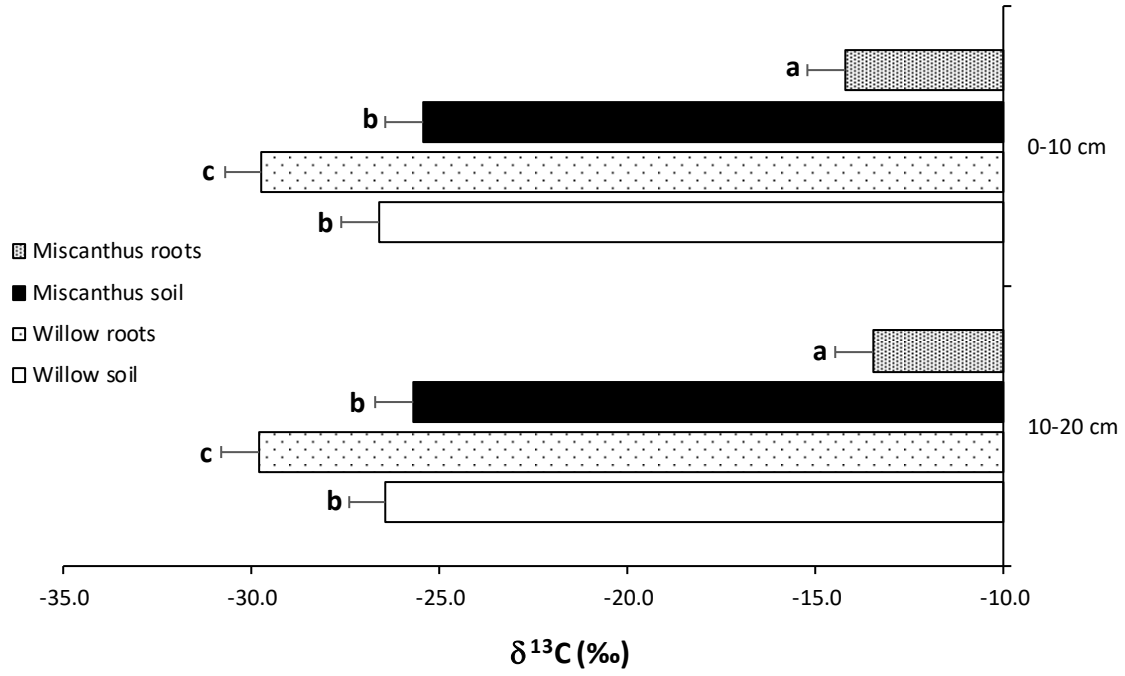

Ac
Ar
Ah
Al
Lr
Lc
Lsp
100%
80%
60%
40%
20%
0%
% of Earthworm species relative to total numbers
Willow
Miscanthus

a


Fungi
Gram(+)
Gram(-)
Unspecified
100%
80%
60%
40%
20%
0%
% of PLFA groups relative to total PLFA concentrations
Willow
Miscanthus

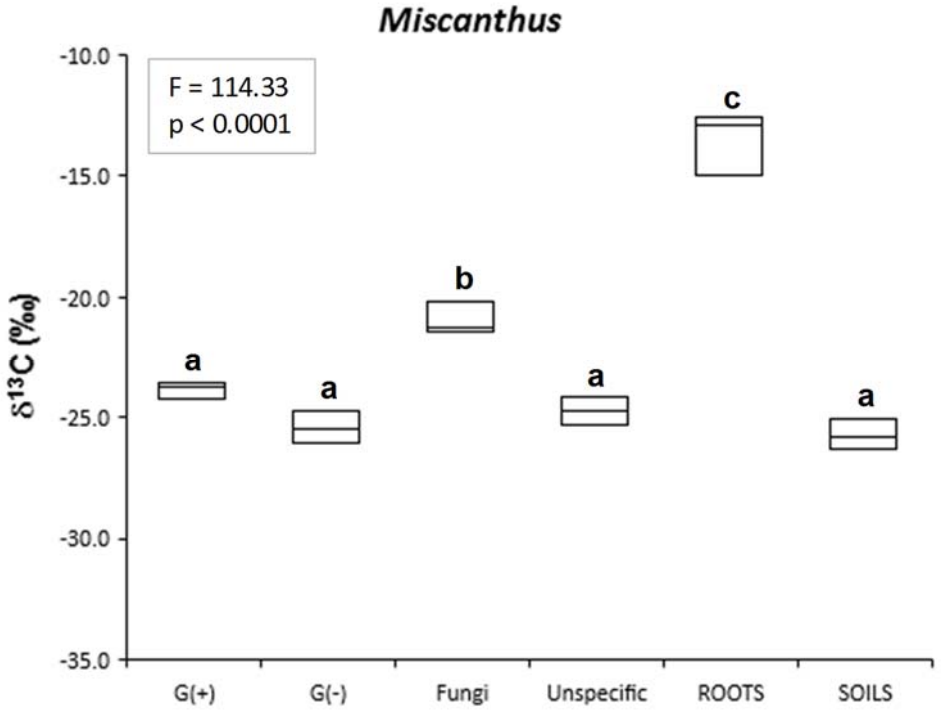
b

9 **Figure 4**


a
b
c
b
0-10 cm
Miscanthus roots
Miscanthus soil
Willow roots
Willow soil
a
b
c
b
10-20 cm
-35.0
-30.0
-25.0
-20.0
-15.0
-10.0
δ13C (‰)

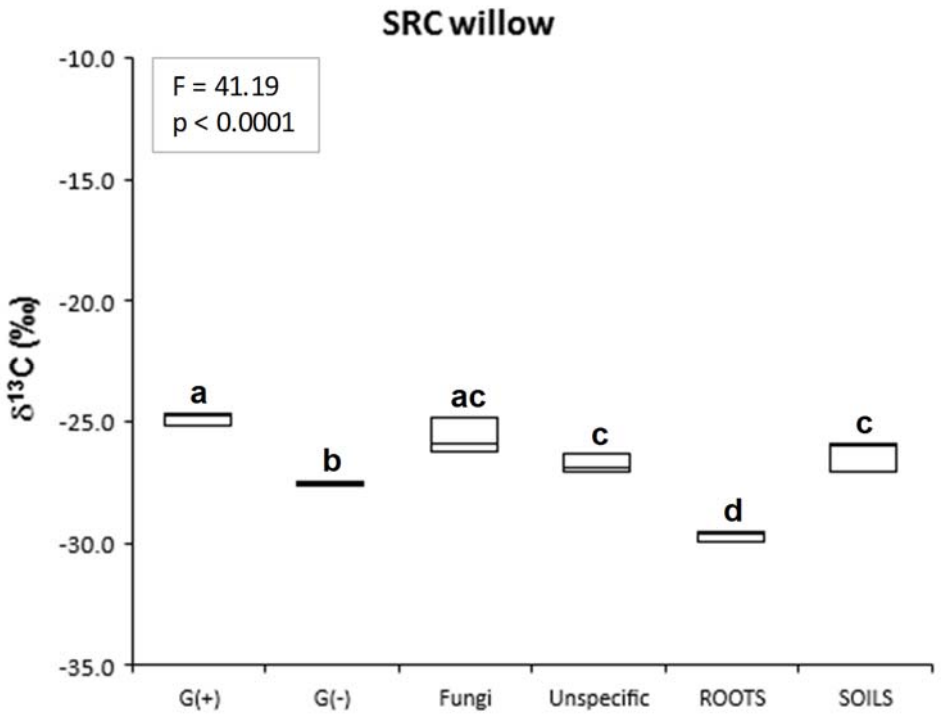
10

11 **Figure 5**



a

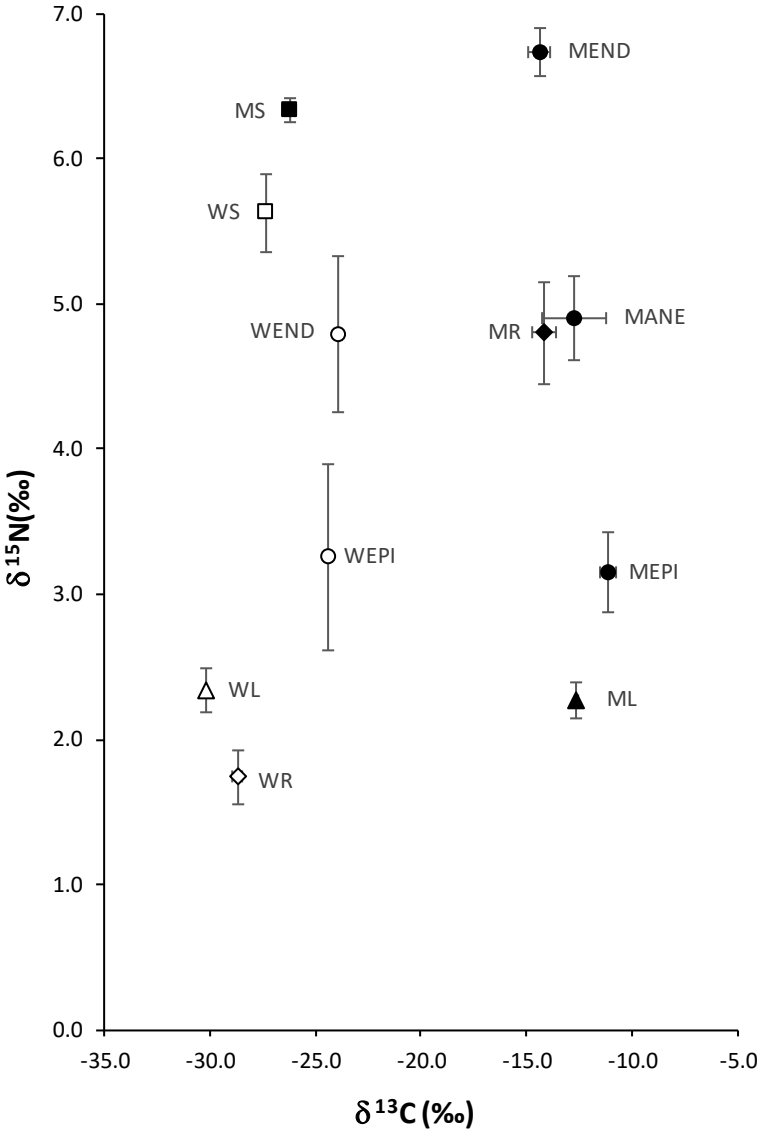
12



b

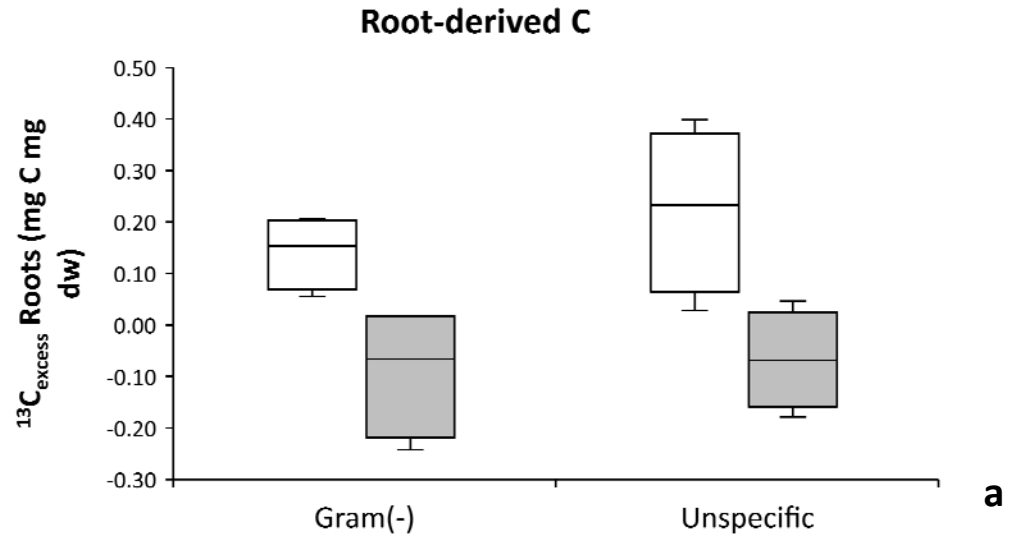
13

14 **Figure 6**

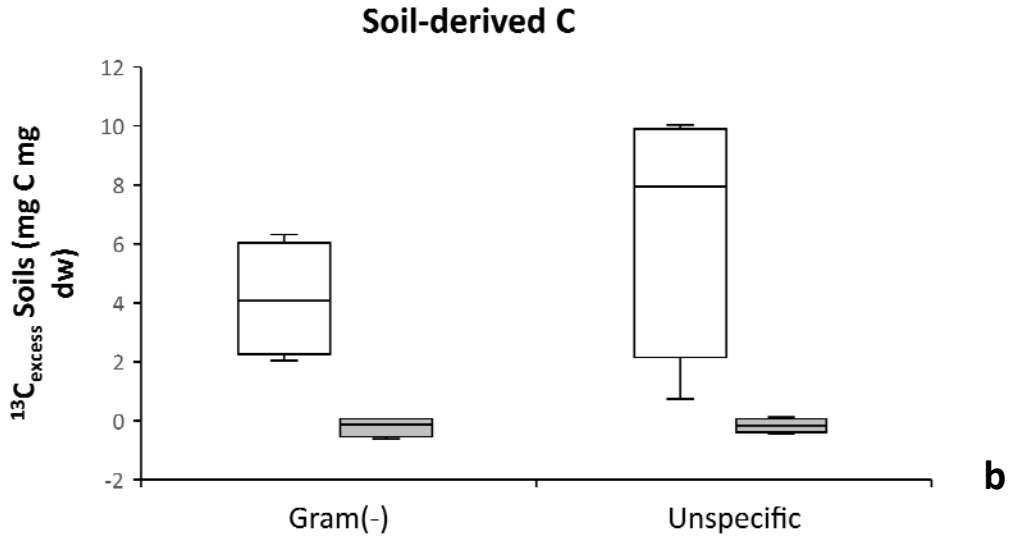


15

16 **Figure 7**

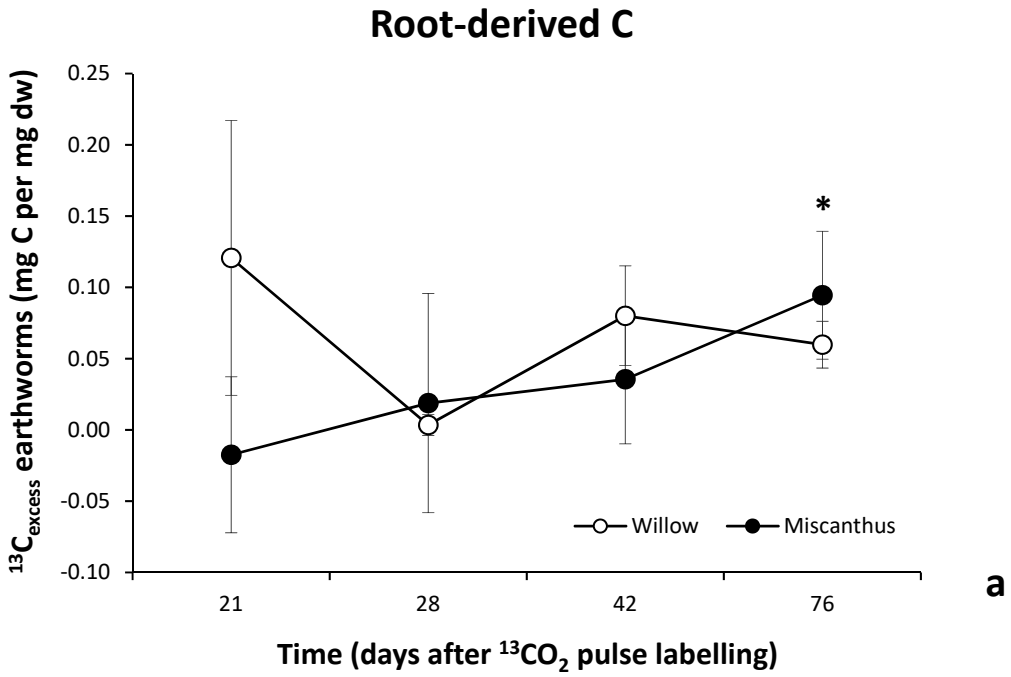


17

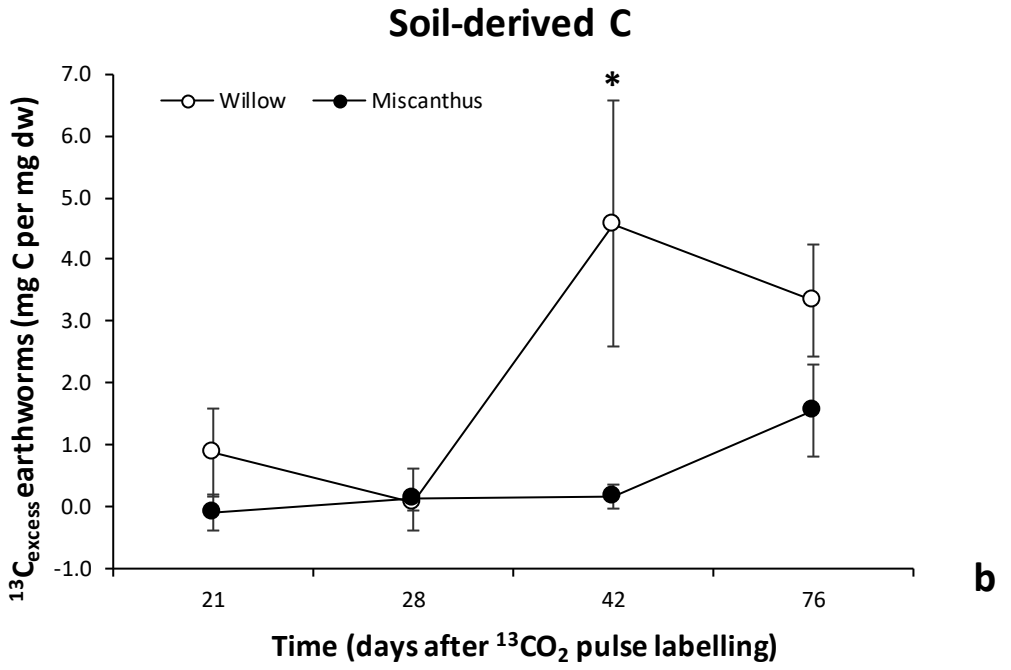


18

Figure 8


Root-derived C
$^{13}C_{excess}$ earthworms (mg C per mg dw)
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
*
Willow
Miscanthus
21
28
42
76
Time (days after $^{13}CO_2$ pulse labelling)

a


Soil-derived C
$^{13}C_{excess}$ earthworms (mg C per mg dw)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
*
Willow
Miscanthus
21
28
42
76
Time (days after $^{13}CO_2$ pulse labelling)

b