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Changes in isotopic signatures of soil carbon and CO_2 respiration immediately and one year after *Miscanthus* removal

JULIA DREWER[1], KARINE DUFOSSÉ[2], UTE M. SKIBA[1] and BENOÎT GABRIELLE[2]
[1]*Centre for Ecology and Hydrology, Bush Estate, Penicuik EH26 0QB, UK,* [2]*AgroParisTech, INRA, UMR 1091 Environnement et Grandes Cultures, 78850 Thiverval-Grignon, France*

Abstract

The removal of perennial bioenergy crops, such as *Miscanthus*, has rarely been studied although it is an important form of land use change. *Miscanthus* is a C4 plant, and the carbon (C) it deposits during its growth has a different isotopic signature ($^{12}/^{13}$C) compared to a C3 plant. Identifying the proportion of C stored and released to the atmosphere is important information for ecosystem models and life cycle analyses. During a removal experiment in June 2011 of a 20-year old *Miscanthus* field (Grignon, France), vegetation was removed mechanically and chemically. Two replicate plots were converted into a rotation of annual crops, two plots had *Miscanthus* removed with no soil disturbance, followed by bare soil (set-aside), one control plot was left with continued *Miscanthus* cultivation, and an adjacent field was used as annual arable crops control. There was a significant difference in the isotopic composition of the total soil C under *Miscanthus* compared with adjacent annual arable crops in all three measured soil layers (0–5, 5–10 and 10–20 cm). Before *Miscanthus* removal, total C in the soil under *Miscanthus* ranged from 4.9% in the top layer to 3.9% in the lower layers with δ^{13}C values of −16.3 to −17.8 while soil C under the adjacent arable crop was significantly lower and ranged from 1.6 to 2% with δ^{13}C values of −23.2. This did not change much in 2012, suggesting the accumulation of soil C under *Miscanthus* persists for at least the first year. In contrast, the isotopic signals of soil respiration 1 year after *Miscanthus* removal from recultivated and set-aside plots were similar to that of the annual arable control, while just after removal the signals were similar to that of the *Miscanthus* control. This suggests a rapid change in the form of soil C pools that are respired.

Keywords: bioenergy, CO_2, *Miscanthus*, removal, soil C, stable isotopes

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Introduction

There is increasing demand of growing bioenergy crops to meet the renewable energy quota of 20% from lignocellulosic feedstock by 2020 set by the European Commission (European Parliament, 2009). The C4 grass *Miscanthus* × *giganteus* which is a perennial rhizomatous grass native to Asia has promising potential for considerable biomass production even under cooler climates (Lewandowski *et al*., 2000) and is therefore widely grown in Europe. There have been several studies on management, productivity and harvest (Jørgensen *et al*., 1997; Beuch *et al*., 2000; Kahle *et al*., 2001) but only recently studies on the impact of *Miscanthus* on greenhouse gas emissions (Hillier *et al*., 2008; Don *et al*., 2012; Drewer *et al*., 2012) and soil carbon (Hansen *et al*., 2004; Lemus & Lal, 2005; Brandao *et al*., 2011) emerged. It has been suggested that *Miscanthus* is sequestering carbon (Clifton-Brown *et al*., 2007; Anderson-Teixeira *et al*., 2009; Dondini *et al*., 2009a; Brandao *et al*., 2011) although the rate might be highly variable. However, more data of C-sequestration under *Miscanthus* in European climates are still needed (Hansen *et al*., 2004) to assess the potential long-term benefit. Including annual harvests, *Miscanthus* can be grown long term up to 20–25 years (Beuch *et al*., 2000) although there are no binding guidelines for farmers. The lifespan of *Miscanthus* might be extended by application of fertilizer to keep yields viable for longer (Danalatos *et al*., 2007; Cadoux *et al*., 2012). However, at some point, it will not be feasible anymore to keep a *Miscanthus* plantation. Little is known of the environmental consequences of the inevitable removal of *Miscanthus* plantations (Dufossé *et al*., 2014), in particular changes in soil carbon storage and greenhouse gases fluxes during the actual removal process and for the land use thereafter. To date, we do not

Correspondence: Julia Drewer, NERC Centre for Ecology and Hydrology, Bush Estate, Penicuik EH26 0QB, UK, tel. +44 131 4454343, fax +44 131 4453943, e-mail: juew@ceh.ac.uk

know enough about conversion from perennial bioenergy crops to arable or fallow, which might be different from conversions of other land uses, to make robust assumptions.

Miscanthus is a C4 plant, and the carbon (C) it has deposited over its ~20 years of growth will have a different isotopic signature ($^{12}/^{13}$C) compared to a C3 plant (Hansen *et al*., 2004). The preference for ^{12}C isotope results in a depletion of ^{13}C in plant biomass in relation to the atmosphere (Balesdent *et al*., 1987; Hansen *et al*., 2004), this will be different in C3 and C4 plants and therefore provides a useful tool to study C turnover in soils where C3 plants (e.g. wheat and barley or grassland) have been replaced by C4 plants like *Miscanthus*. Hence, *Miscanthus* as a C4 plant is expected to have a higher ^{13}C abundance than traditional C3 plants (Zimmermann *et al*., 2014), which should be recognizable in the total soil C and respiration after decomposing litter from this plant was incorporated into the soil.

By studying the isotopic composition of the total soil C content before and after harvest and comparing it with an adjacent field predominately cultivated with C3 crops, the proportion of C stored and released to the atmosphere by newly sequestered and old carbon can be estimated. With *Miscanthus* as the only C4 source, the isotopic signal can be used to quantify the amount of carbon derived by this energy crop (Balesdent & Balabane, 1992). Humus formation and microbial mineralization induce only slight variations in ^{13}C abundance, hence in cold and temperate climates, δ^{13}C values of soil organic matter range from −24 to −29‰ (Balesdent *et al*., 1987).

Miscanthus has the potential to improve carbon stocks, especially when planted on formerly tilled land (Smith, 2004; Rowe *et al*., 2009). However, little is known about change in carbon stocks and fluxes when land is returned to conventional annual arable use after the lifespan of perennial bioenergy crops. Re-instating annual arable agriculture requires mechanical or chemical removal of the bioenergy crop following mechanical management operations to reseed an annual crop although currently there are no direct guidelines for farmers.

The removal of perennial bioenergy crops, such as *Miscanthus*, has rarely been studied although it is an important form of land use change and essential information for carbon footprint and life cycle analyses. To date, life cycle analyses use estimates rather than measured data of bioenergy crops removal or do not include it at all (Gabrielle *et al*., 2014).

In order to help to close the gap in uncertainties about the end of a bioenergy crop lifespan and land use change back to fallow or recultivation, we have studied changes and isotopic composition of total C in the soil and CO_2 respired during a removal experiment in June 2011 from a *Miscanthus* field cultivated since 1990 in Grignon (France) and 1 year after the removal from recultivated plots and continuous *Miscanthus* cultivation as well as continuous annual arable rotations.

Materials and methods

The investigated site is located in Grignon, 40 km south-west of Paris (48°51′N, 1°58′E) in a degraded oceanic climate zone (Köppen classification) with a mean annual temperature of 11.5 °C and a mean annual precipitation of 557 mm over a 20-year period (1992–2012) (Dufossé *et al*., 2014). The soil texture is a silty clay loam (UDSA Soil Taxonomy) classified as Agrudalf (USDA Soil Taxonomy) or Luvisol. The clay (< 2 μm), silt (2–50 μm) and sand (50–2000 μm) fractions in the 0–15 cm topsoil layer are 33%, 50% and 17% (dry weight basis), respectively.

The *Miscanthus* field was established in June 1990 with dimensions of 6 m by 50 m and is adjacent to a field cultivated with annual arable crops. Typical crop rotations included wheat, barley and maize. The *Miscanthus* stand was planted from rhizomes and saplings, with a density of two plants m^{-2}. Some rhizomes were replanted during the following year to maintain an even plant density. Harvest generally took place in late February or early March when the moisture content of the biomass dropped below 20%. A more detailed description of the site details, agricultural management and changes in GHG fluxes during the *Miscanthus* removal can be found in Dufossé *et al.* (2014). In this work, we only report isotopic composition of C in the soil and CO_2 respired measured before and after *Miscanthus* removal in June 2011 and 1 year later. Aboveground biomass was chopped and ground in late June, during the peak growing season to weaken the rhizomes. Then, in late August, at the onset of leaf senescence, when N is remobilised to the rhizomes, glyphosate was sprayed. In mid-October, two replicate plots (A and E) were tilled and converted into a rotation of annual arable crops with wheat in the first year and two plots (B and D) had *Miscanthus* removed with no soil disturbance, followed by bare soil (set-aside or fallow). Additionally, one plot was left as control with continued *Miscanthus* cultivation (C), and an adjacent field under continuous annual arable rotation (AC) was used as a control for annual arable crops. The layout of the field/plots is shown in Fig. 1. Gas samples for CO_2 respiration were taken before and after the removal and following each management/recultivation operation. A year later, the site was revisited and changes in the isotopic composition (^{13}C/^{12}C) of total soil carbon and respired CO_2 measured again.

Soil sampling

In June 2011, soil samples were taken with a Dutch auger at three different depths, 0–5, 5–10 and 10–20 cm. Ten replicate samples were taken for each depth for all treatments, *Miscanthus* control (MC later C), *Miscanthus* removed (MR, later A, B, D, E) and the arable control (barley, AC). As samples were taken before the removal, *Miscanthus* control and *Miscanthus*

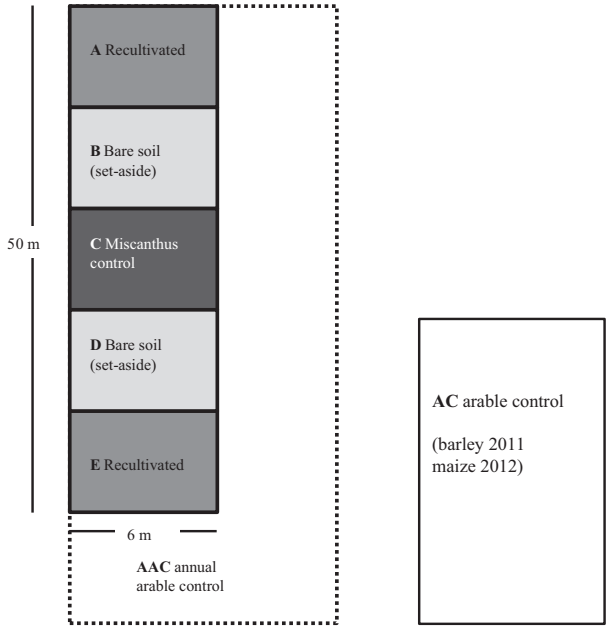

Fig. 1 Experimental design after *Miscanthus* removal, solid line including A–E previously (until June 2011) *Miscanthus* cultivation, after removal A and E recultivated (wheat), B and E bare soil (set-aside) and C continued *Miscanthus* cultivation, AAC annual arable control surrounding the *Miscanthus* plots (wheat, only sampled in 2012) and AC as adjacent field arable control (barley in 2011, maize in 2012).

removed were essentially the same treatment, after the removal operations, the plots were converted into the different treatments.

One year after the removal, in June 2012, the 2011 sampling strategy was repeated with eight soil samples taken per treatment, namely arable control (AC – maize), *Miscanthus* control (C), recultivated (A and E) and bare soil (B and D) from three different depths (0–5, 5–10 and 10–20 cm), respectively. Additionally, four samples at three depths were taken from the annual arable control surrounding the *Miscanthus* field (AAC – wheat) because the arable control field (AC) which was barley in the previous year was now cultivated with the C4 crop maize. Also, the arable control surrounding the *Miscanthus* field (AAC) was essentially the same treatment as the recultivated plots (A and E) with the difference that it had been in an arable rotation throughout while the recultivated plots were under *Miscanthus* for the previous 21 years.

The soil was oven-dried at 105 °C, then ball milled to a fine powder for analysis. Soil samples were analysed for $\delta^{13}C$ at CEH Lancaster using Eurovector EA – Isoprime IRMS. Ground, dried soil samples were weighed into tin capsules and combusted using a Eurovector elemental analyser. Resultant CO_2 from combustion was analysed for $\delta^{13}C$ using a Micromass Isoprime IRMS. Standard deviation for the $\delta^{13}C$ for quality control and duplicate samples was not more than 0.13‰. Standard deviation for the percentage total carbon for the quality control and duplicate samples was not more than 0.86%.

Ecosystem respiration CO_2 gas sampling

Opaque manual static chambers were installed in June 2011. They consisted of square aluminium frames of 49-cm length and 30-cm height, which were pushed into the ground to a depth of 10 cm. Initially, five chambers were installed in the *Miscanthus* control and five each on either side of the control plot which would become the *Miscanthus* removal plots. Additionally, five chambers were installed in the barley control on the adjacent field. For the duration of the removal operations, only the *Miscanthus* control chambers stayed in situ, the others were taken out, and after all management operations, three chambers were randomly placed in the recultivated plots A and E and bare soil plots B and D, respectively. They were only removed briefly before soil tillage or harvest, and inserted back immediately after. As above-ground vegetation remained in the chambers combined autotrophic and heterotrophic, CO_2 fluxes were measured and it will be referred to as ecosystem or CO_2 respiration rather than soil respiration. For gas sampling, white alveolar PVC lids were sealed to the chambers with neoprene sponges and clips. Chamber air was drawn out of the sealed chambers through a septa situated in the middle of the lid using syringe needles. Chamber air was sampled 0, 15, 30 and 40 min after closure using a 20-mL syringe. Pre-evacuated exetainers© (Labco, Lampeter, UK) of 12 mL were filled with 18 mL of headspace gas. Gas samples were taken 2 days before the removal as a background, then on the day of removal and 1, 2, 4 and 6 days after the removal operation. In the autumn, samples were taken before chiselling, then after chiselling/before ploughing and after ploughing.

One year after the *Miscanthus* removal, gas samples were taken from all chambers once in June 2012 as described above. At this time, there were five chambers in the Miscanthus control (C) and arable control (AC: maize following barley in 2011), respectively, and three chambers each in the recultivated (A and E), bare soil (B and D) and additionally surrounding annual arable crop (AAC – wheat), respectively.

An accredited method (Reference SOP-2105) was used at CEH Lancaster to determine the stable isotopes in ecosystem respiration (CO_2). Gas samples were injected into the trace gas preconcentrator using a gas tight syringe. Water was eliminated via a perchlorate chemical trap and the CO_2 cryogenically preconcentrated prior to gas chromatography column separation and introduction to a Micromass Isoprime IRMS via open split. The $\delta^{13}C$ was measured and expressed in ‰ (vs. PDB).

Concentrations of CO_2 were analysed at CEH Edinburgh on an HP5890 Series II gas chromatograph (Hewlett Packard (Agilent Technologies) UK Ltd., Stockport, UK) with flame ionization detector and methaniser. The limit of detection for CO_2 was 19 ppm. Samples were analysed within 2 weeks during which storage loss is typically negligible.

Keeling plots were derived for each measurement occasion per chamber for which $\delta\ ^{13}C$ was plotted vs. $1/CO_2$ concentration (ppm) (Pataki *et al.*, 2003) to derive the source partitioning. With $\delta\ ^{13}C$ on the *y*-axis and the inverse CO_2 concentration on the *x*-axis, the intercept on the *y*-axis determines the $\delta\ ^{13}C$ of the source. This enabled the comparison of the different chambers and their sources.

For Keeling plots and mixed effects models, the software package R (R Development Core Team, 2011) was used as well as MINITAB® 16.2.4 for ANOVA.

Results

Soil carbon

Compared with the annual food crops (AC), total C was a significantly higher ($P < 0.001$) in soil under the *Miscanthus* plots (MR and MC), in all three layers in 2011, the year of removal. As expected, there was no significant difference between the *Miscanthus* control and removal plots. Furthermore, there was a significant ($P < 0.001$) difference in the isotopic composition of the total soil C under *Miscanthus* (MR and MC) compared with adjacent annual arable crops (AC) in all three measured layers (0–5, 5–10 and 10–20 cm) but not between the control and removal plots (MC and MR). In the year of the *Miscanthus* removal, total C in the soil under *Miscanthus* ranged from 4.9% in the top layer to 3.9% in the lower layers with $\delta^{13}C$ values of −16.3‰ to −17.8‰ while soil C under the adjacent arable crop ranged from 1.6% to 2% with $\delta^{13}C$ values of around −23.2‰ (Fig. 2). Accordingly, the differences in total C and $\delta^{13}C$ were very clear between the *Miscanthus* and annual arable crops ($P < 0.001$).

One year later, the soil under continued *Miscanthus* cultivation (C) had 4.2% C in the top layer to 3.2% in the lower layers with $\delta^{13}C$ values ranging from −15.4‰ to −17.2‰ (Fig. 3). Removal plots now under cultivation (A and E) or left bare (B and D) still had similar total C and $\delta^{13}C$ values to the *Miscanthus* control while the adjacent arable plots had lower total C and $\delta^{13}C$ values as measured in the previous year (Fig. 3). Total C from former (now recultivated or left bare) and current *Miscanthus* (control) plots were significantly different ($P < 0.05$, mixed effects model with plot as random effect, crop and depths as fixed effects) from both arable controls (AC and AAC). However, differences within the *Miscanthus* plots, namely control (C), recultivated (A and E) and left bare (B and D) were not significant. There was also no significant difference between the *Miscanthus* and former *Miscanthus* plots in terms of $\delta^{13}C$. Differences in $\delta^{13}C$ between the *Miscanthus* control (C), recultivated (A and E), bare (B and D) and the adjacent annual arable plot (AC) were significant ($P < 0.001$), but the difference between the current and former *Miscanthus* plots and the surrounding AAC plot was not significant in terms of $\delta^{13}C$. The significant differences were found between crop/management types, and there were no significant differences between the plots only, so that A and E, and B and D can be regarded as replicates of the same treatment, namely A and E recultivated and B and D bare soil, as intended.

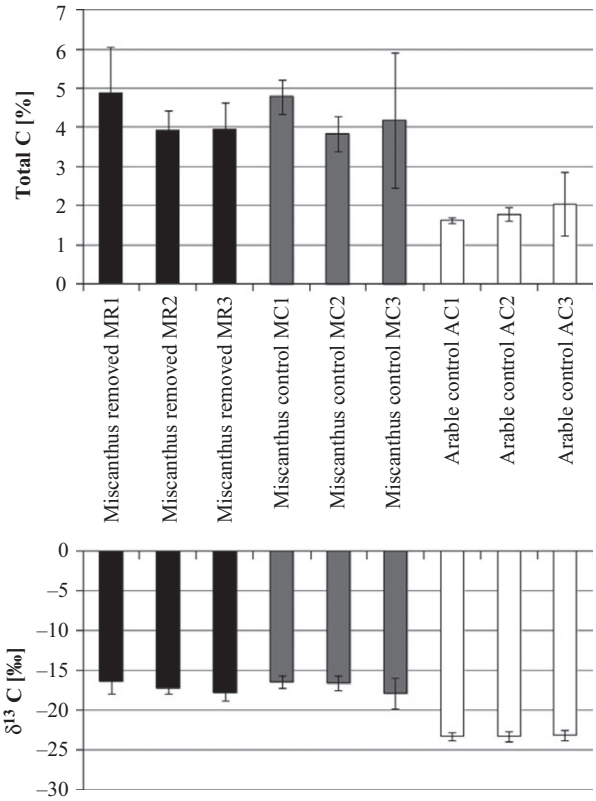

Fig. 2 Total carbon (C) in % and $\delta^{13}C$ in ‰ in *Miscanthus* removed (MR), *Miscanthus* control (MC) and arable control (AC – barley) plots at three different soil depths, 1 = 0–5 cm, 2 = 5–10 cm and 3 = 10–20 cm in the year of *Miscanthus* removal. Data shown as averages and standard deviation of 10 replicate samples.

CO_2 respiration

Isotopic source signatures (derived from Keeling plots) in the year of *Miscanthus* removal were between −9‰ and −11‰ in the CO_2 respiration from chambers on the *Miscanthus* plots. There were no significant differences between the samples taken before removal and daily during the week after removal (Fig. 4). For comparison with 2012, an average over the whole week was used for statistical analyses. As expected, there were no significant differences between the removal plots and the *Miscanthus* control in 2011. In contrast, isotopic source signatures from the chambers in the adjacent arable control were between −22‰ and −29‰ which was significantly different from the Miscanthus plots ($P < 0.001$). Chiselling and tillage in autumn of 2011 did not result in any short-term changes of CO_2 respiration (results not shown).

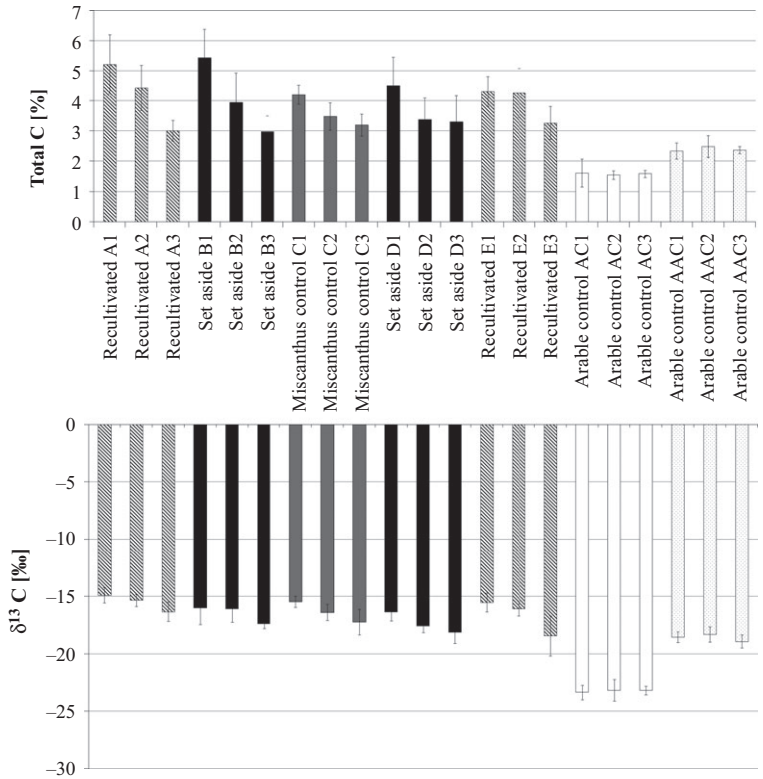

Fig. 3 Total carbon (C) in % and $\delta^{13}C$ in ‰ 1 year after *Miscanthus* removal at three different depths, 1 = 0–5 cm, 2 = 5–10 cm and 3 = 10–20. A and E are recultivated (wheat), B and D are bare soil (set-aside), C is *Miscanthus* control, AC is arable control (maize, previously barley) and AAC is annual arable control surrounding the plots (wheat). Error bars are standard deviation of 8 (C and AC) and 4 (A, B, D, E, AAC) replicate samples.

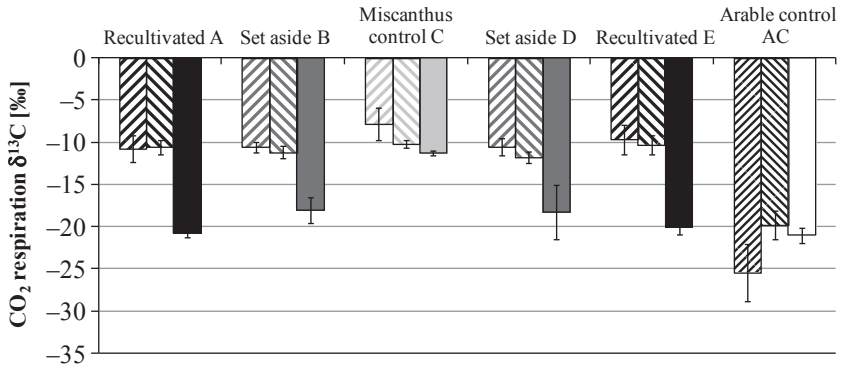

Fig. 4 Isotopic signature derived from Keeling plots ($\delta^{13}C$ vs. $1/CO_2$ conc.) for the different treatments (A and E are recultivated (wheat), B and D are bare soil (set-aside), C is *Miscanthus* control, AC is arable control). Pattern is 2011 (backward slash = before removal and forward slash = the week just after removal), solid fill is 2012, 1 year after removal. Error bars are standard deviation of three replicates per treatment and five replicates for the controls.

One year later, isotopic source signatures from the *Miscanthus* control were around −11‰, −19 to −21‰ from the recultivated plots (A and E), −16 to −19‰ from the bare soil plots (B and D) and −20 to 22‰ from the adjacent arable control (Fig. 4).

In the year of *Miscanthus* removal (2011), the isotopic ratio from CO_2 respiration under *Miscanthus* (MC and MR) was significantly different ($P < 0.001$) from under the arable control AC). The year after *Miscanthus* removal, the isotopic signature of the recultivated plots

(bare and with crop) was similar to the arable control while the signature of *Miscanthus* was the same as in the previous year. In summary, the isotopic signature of CO_2 was significantly different ($P < 0.001$) from the *Miscanthus* control compared to all other crops/management types. There were no significant differences between the former *Miscanthus* plots (A, B, D and E) and the arable control (AC).

Discussion

The clear differences in total soil carbon under *Miscanthus* and under annual arable crops suggest an accumulation of soil C over 20 years of *Miscanthus* cultivation as already found for soil organic matter (Hansen *et al*., 2004; Dondini *et al*., 2009b; Dufossé *et al*., 2014). Dondini *et al*., 2009b also observed in a study in Ireland that top soil layers for both, arable and *Miscanthus* crops, contained more C than lower layers which has also been found in this study in France (also Dufossé *et al*., 2014). As in our case, the annual arable crop rotation included maize as a C4 crop, the proportion of *Miscanthus*-derived carbon (as e.g. in Dondini *et al*., 2009a,b) could not be estimated without bias (because *Miscanthus* was not the only C4 crop) and was therefore not attempted. Furthermore, we are comparing C concentrations rather than C stocks. The focus here was on the change after *Miscanthus* removal and recultivation. Total soil carbon under *Miscanthus* was more enriched in $\delta^{13}C$ than under the arable crops in all measured depths as also reported by Dondini *et al*., 2009b for soil organic matter. It has been reported that $\delta^{13}C$ from soil organic matter under *Miscanthus* decreased with depth (Gregorich *et al*., 1995; Dondini *et al*., 2009b); this trend could not be seen in our study for total soil carbon for any of the crops. However, both studies sampled to greater depths (> 60 cm) with the measured differences not being significant at the 0–20 cm depths. In addition, it has been reported that according to stable isotope ratios, large fractions of the soil organic matter pool under *Miscanthus* were indeed *Miscanthus*-derived carbon (Hansen *et al*., 2004) which would likely be the case in this study, too, because of the large difference in isotopic ratios between the *Miscanthus* and arable plots. Changes in soil organic matter, yields and greenhouse gas fluxes in general are discussed in Dufossé *et al*., 2014.

Our measured values (between −9‰ and −11‰ in the CO_2 respiration from chambers on the *Miscanthus* plots and −22‰ and −29‰ from arable) fit in the ranges of those reported previously (Smith & Epstein, 1971; Balesdent *et al*., 1987), namely that common isotopic composition from atmospheric CO_2 for C3 plants ranges from −23 to −40‰ and from −9 to −19‰ for C4 plants. The transitions from C4 (*Miscanthus*) to C3 (−19 to −21‰ from the recultivated wheat plots (A and E), −16 to −19‰ from the bare soil plots (B and D)) were at the high end of the reported values for C4 and low end of C3 which follows from that.

Studies have investigated soil carbon sequestration and associated ^{13}C signal during the *Miscanthus* establishment phase of *Miscanthus* (Zimmermann *et al*., 2012) or under younger stands (Hansen *et al*., 2004; Dondini *et al*., 2009b), but to date, there is no information on the end of life span or recultivation into different land uses after *Miscanthus* cultivation and subsequent removal. Our study added some (limited) information on soil carbon and associated isotopic signature short term (1 year) after removal.

One year after *Miscanthus* removal, total soil carbon was actually higher (by about 1% in the top layer) in recultivated and bare soil plots than the *Miscanthus* control, even if not significantly. Bare soil plots had some regrowth of *Miscanthus* amongst other weeds. However, as the isotopic composition of the recultivated and bare soil plots was not different from the *Miscanthus* control, we can conclude that changes in total soil carbon after *Miscanthus* removal do not occur after a short time scale of 1 year.

Hence, measurements in the following years will be important to assess the change and rate of change of soil C in the field converted from *Miscanthus* to annual arable food crops and make predictions into the future. In contrast, the isotopic signature of CO_2 respiration did change 1 year after *Miscanthus* removal for the recultivated and bare soil plots, which now had a signature closer to that of the arable control than the signature of the *Miscanthus* control. So, the C respired in 2012 was mostly derived from the crops established in 2011 (wheat) or the weeds on the bare plots.

This suggests that the change in the type of carbon pool that is respired occurs more rapidly than the variations in total C content. Thus, the carbon sequestered under a long-term *Miscanthus* cultivation may be only slowly released, and after removal, the carbon respired may come from sources with higher turnover rates or more recent litter decomposition, in particular from the crop following *Miscanthus*.

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