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Carbon Inputs from *Miscanthus* Displace Older Soil Organic Carbon Without Inducing Priming

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Abstract The carbon (C) dynamics of a bioenergy system are key to correctly defining its viability as a sustainable alternative to conventional fossil fuel energy sources. Recent studies have quantified the greenhouse gas mitigation potential of these bioenergy crops, often concluding that C sequestration in soils plays a primary role in offsetting emissions through energy generation. *Miscanthus* is a particularly promising bioenergy crop and research has shown that soil C stocks can increase by more than 2 t C ha^{-1} yr^{-1}. In this study, we use a stable isotope (^{13}C) technique to trace the inputs and outputs from soils below a commercial *Miscanthus* plantation in Lincolnshire, UK, over the first 7 years of growth after conversion from a conventional arable crop. Results suggest that an unchanging total topsoil (0–30 cm) C stock is caused by *Miscanthus* additions displacing older soil organic matter. Further, using a comparison between bare soil plots (no new *Miscanthus* inputs) and undisturbed *Miscanthus* controls, soil respiration was seen to be unaffected through priming by fresh inputs or rhizosphere. The temperature sensitivity of old soil C was also seen to be very similar with and without the presence of live root biomass. Total soil respiration from control plots was dominated by *Miscanthus*-derived emissions with autotrophic respiration alone accounting for ~50 % of CO_2. Although total soil C stocks did not change significantly over time, the *Miscanthus*-derived soil C accumulated at a rate of 860 kg C ha^{-1} yr^{-1} over the top 30 cm. Ultimately, the results from this study indicate that soil C stocks below *Miscanthus* plantations do not necessarily increase during the first 7 years.

Keywords Soil C · Priming · Bioenergy · 13CO2 · Greenhouse gas · Autotrophic soil respiration

Introduction

The most trusted predictions for the impacts of anthropogenic climate change suggest an increase in global average temperatures that will have a wide range of detrimental effects on human and natural systems [1, 2]. This climate change is primarily driven by increasing concentrations of greenhouse gases (GHGs) in the atmosphere, and in particular carbon dioxide (CO_2) emissions resulting from burning fossil fuels for energy generation [3]. Bioenergy crops like *Miscanthus* have the potential to displace some of our dependency on non-renewable fossil fuels [4, 5] but the relative advantage of bioenergy is largely influenced by realised yields and carbon (C) sequestration in pools that remain after the aboveground biomass has been harvested [6, 7]. Even a relatively modest 1 t C ha^{-1} yr^{-1} [8] increase in soil C stocks can improve the GHG footprint of *Miscanthus* by 314 g CO_2-eq kWh^{-1}, assuming electricity generation in a steam-turbine power station averaging 30 %

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efficiency [9] and an average harvested yield of 10 t ha^{-1} yr^{-1} [10, 11] (Eq. 3 [7]). While there are a number of models capable of accurately simulating yields for a wide range of conditions (e.g. Clifton-Brown et al. [12]), predicted changes in soil C stocks are less certain, and for the less studied crops like *Miscanthus*, uncertainty is particularly high [13, 14].

Previous research has suggested that *Miscanthus* can sequester more than 2 t C ha^{-1} yr^{-1} in the top 30 cm of soil [15] but a site in Lincolnshire, UK, noted no change 7 years after the plantation was established [7]. Consequently, it is essential to better understand the reasons that explain why some soils, and plantations, show large increases in soil C stocks [15] and others show no significant change [8, 16], or even decreases [13, 16]. Further, to allow accurate model simulations of temporal and spatial scales beyond those practical to measure, we must better understand the mechanisms that underpin soil C sequestration and soil organic matter (SOM) decomposition. Since these findings tend to be very site-specific, many studies are required to evaluate the potential of soil C storage below bioenergy crops.

Fluxes in and out of soil C pools are predominantly due to decomposition, a process resulting in CO_2 emissions from the breakdown of organic matter. Senescence of plant matter typically provides the majority of this organic matter, and in productive systems such as *Miscanthus* this can be a large annual C addition [15, 17]. However, the addition of fresh organic matter has been observed to stimulate increased decomposition of older soil C through a process known as priming [18, 19]. This can offset the fresh C additions and has the potential to break down more stable forms of soil C, reducing the longevity of total soil C stocks [20]. Consequently, it is important to quantify both decomposition of new C additions and older soil C separately. However, this is particularly difficult as they cannot be physically isolated in a practical way. Advances in isotopic analysis have provided an effective solution to this problem, exploiting differences in naturally occurring isotopic differences [21] or artificially introducing a unique concentration of ^{13}C or ^{14}C isotopes [22].

Miscanthus grown in most temperate zones provides an ideal model for isotopic partitioning of new and old C; the natural abundance of ^{13}C isotopes in *Miscanthus* biomass (a C4 crop) is different to the ^{13}C abundance in soils created from C3 plant matter [23]. This allows pre-existing C to be quantified separately to the *Miscanthus*-derived C, in both CO_2 emissions and soil C. Using this approach, quantifying the *Miscanthus*-derived C in soil is relatively simple and repeated sampling allows for simple linear rates of net *Miscanthus* C sequestration to be estimated [8]. While quantifying the C4 and C3 soil C pools represents the net change, it does not differentiate between decomposition of old and new additions: how much of the fresh additions are retained? Ultimately the answer to this is site specific since decomposition, which drives the majority of losses, is governed by a wide range of abiotic and biotic factors [24]. Consequently, if we are to predict the net impacts of a *Miscanthus* plantation on soil C stocks, we must also define accurate relationships between climatic variables and decomposition.

While biological activity and the quality of organic matter [25] are arguably the most important factors governing decomposition of plant matter into soils, temperature is also often cited (e.g. Davidson and Janssens [26]) as a key driver. In light of climate change likely to increase global temperatures, the temperature sensitivity of soil C decomposition is a major issue; globally, soils contain an estimated 2300 Gt C [27]. In upland mineral soils alone, predicted losses due to a climate change related increase in decomposition are ~40 Gt C by 2100 [28], meaning a substantial addition of CO_2 to the atmosphere, on top of those already occurring, further contributing to climate change. Consequently, there is a potential for soil C sequestration under bioenergy crops like *Miscanthus* to play a key role in mitigating some of these losses, but the effects of temperature on decomposition rates (and therefore CO_2 emissions) must also be taken into account. It is worth noting that many models assume decomposition of recent C additions is just as sensitive to temperature as decomposition of older SOM [29–31]. However, this is not always accurate [32–35]. Therefore further emphasising the importance of understanding the influence of new crops and their C additions on SOM decomposition.

Measuring the CO_2 emissions resulting from SOM decomposition as opposed to other sources of soil respiration (e.g. autotrophic respiration, R_a) can be difficult in situ. As a result, the temperature sensitivity of SOM decomposition is most commonly evaluated under laboratory conditions, with soils collected from a range of locations and incubated at progressively higher temperatures [36, 37]. While this is particularly effective at isolating the temperature effect from other confounding variables, and therefore creating realistic Q_{10} values [36, 38], it is not always an accurate depiction of responses under field conditions [39]. Consequently, isotopic techniques have been used to partition soil respiration and isolate SOM decomposition in situ, thereby including important interactions with more realistic biotic conditions [21, 40]. To date, no studies have explicitly linked SOM decomposition to temperature under *Miscanthus* plantations but Zatta et al. [41] observed the displacement of C3-derived (pre-*Miscanthus*) C with C4-derived (sourced from *Miscanthus* only) additions. Although no direct assessment was made, this study hypothesised that the displacement was due to a priming effect on SOM decomposition.

The occurrence of priming is typically attributed to the interactions between live or fresh C inputs and older SOM, accelerating decomposition through microbial activity [42, 43]. The excess decomposition, and therefore priming effect, is often measured through additional CO_2 respired and/or changes in C stocks [43, 44]. The mechanisms behind the

priming effect have proven difficult to quantify [18, 44, 45] but studies suggest that fresh organic matter increases the availability of labile C and therefore increases microbial activity (and decomposition) [42]. Subsequently, the increased microbial decomposition can exhaust available nutrients and when this occurs, more stable soil C compounds incur larger losses [19]. As long as additions exceed losses, there will be a net C sequestration in soils, but it is important to quantify losses and gains separately as the stability of soil C compounds is just as important as the overall balance.

This study aims to partition soil respiration fluxes and soil C pools into *Miscanthus*-derived and 'old soil' (C3 soil - pre-*Miscanthus*) sources. Using stable isotope (^{13}C) analysis to quantify the relative contribution of *Miscanthus* to CO_2 losses and soil C gains, we present estimated rates of additions and losses from a 7-year-old commercial plantation in Lincolnshire, UK. A comparison of undisturbed controls and bare plots with no new C additions was used to estimate the effects of priming due to fresh *Miscanthus* additions. Given early indications of unchanging total soil carbon stocks, we postulated that annual *Miscanthus*-derived C additions would equal the annual losses from C3-derived SOM decomposition, therefore creating a net balance of soil C. Further, we hypothesised that losses from C3 soil stocks would be greater in control plots than in bare plots due to a priming effect.

Materials and Methods

Study Site

The field experiment was conducted in a commercial (11.5 hectares, ha) *Miscanthus* plantation near Lincoln, Lincolnshire, UK. The soil type is a compacted loam that behaves like a heavy clay, with approximately 15, 36 and 49 % of, respectively, clay, silt and sand in the top 30 cm of soil. The top 30 cm of soil had a mean total C and N concentration of, respectively, 1.86 and 0.18 %, with a soil pH ranging from 6.8 to 7.3. The bulk density of the soil was 1.46 ± 0.03 g cm^{-3} for the 0–15 cm layer and 1.53 ± 0.02 g cm^{-3} for the 15–30 cm soil layer. The deeper soil profile showed an increasing bulk density (1.59 ± 0.20 g cm^{-3}, 30–50 cm; 1.62 ± 0.10 g cm^{-3}, 50–100 cm) and a clear B-horizon at the plough depth (30 cm). The site had a mean annual temperature of 9.9 °C and a mean annual precipitation of 605 mm (30-year average 1980–2009) with 300 mm falling between May and October. *Miscanthus* was established in 2006 at a density of 10,000 rhizomes ha^{-1}. The crop was harvested annually in March, beginning in 2008, with biomass removal carried out only from 2009 onwards; yields (with 20 % moisture content) averaged 7.58 dry t ha^{-1} for 2009 to 2013, inclusive. Harvest in 2011 was pushed back to mid-April due to heavy rains in late March but no noticeable re-sprouting had occurred at the time of harvest in any year. Since planting, the *Miscanthus* plantation was only fertilised once in 2010, with phosphorus and potassium (Fibrophos, 660 kg ha^{-1}). Land management prior to conversion to *Miscanthus* was a rotation of winter wheat and oilseed rape, with 3 years of wheat directly before conversion. Discussions with the landowners noted that the land use history of this field was arable cropping of C3 crops (specifically wheat, oil seed rape, barley or rye) for at least 30 years. More details about the soil and site management can be found in Robertson et al. [7].

Experimental Design and Environmental Variables

During the winter of 2008/2009 (before February 2009), five 15 m^2 areas (blocks) within the plantation were randomly selected to host 10 treatment plots: one undisturbed control and one bare soil plot within each block (Fig. S1). Plots within each block were at least 5 m from each other and both controls and bare plots were marked out to be 1.6 m diameter circles (2 m^2). At the time of creating these plots, the *Miscanthus* was still spreading to fill in gaps between plants; patchiness is common for *Miscanthus* stands, with Zimmermann et al., [46] reporting the average gap size as 3.67 m^2 in three or four year old commercial plantations. Therefore, no plant removal was necessary to establish all plots between plants, and consequently bare plots contained no established (i.e. living) rhizomes. The bare soil plots were prepared by trenching a 1.6 m diameter circle centred on a PVC chamber designed for CO_2 measurements as described below. Trenches were 70 cm deep and lined with a double layer of thick polyethylene to exclude future root propagation; inspection for lateral root growth into the trenched plots revealed little evidence of *Miscanthus* roots but there was likely some that remained undetected. Although some studies report *Miscanthus* to be very deep rooting [47], the extent of this propagation is heavily influenced by the soil type [48]. Indeed, Monti and Zatta [49] noted that in a 5-year old plantation, almost 90 % of all roots were in the top 35 cm and less than 0.5 % of root dry weight was below 75 cm. Consequently, 70 cm was deemed sufficient at this site, particularly because the soil became heavily compacted at this depth and at the time of trenching there was no visible evidence of plant biomass. After trenching, there was no living *Miscanthus* biomass in the soil of the bare plots, and any later root growth below the impermeable liner was assumed to have negligible contributions to the fluxes and pools measured by this study. In addition to trenching to exclude root growth under bare plots, the aboveground litter layer was carefully removed to expose bare soil but also to limit disturbance. After clearing the plots, a 20 mm^2 mesh screen was placed over the plots to ensure the soil surface was kept clear of *Miscanthus* litter. During each monthly visit to the site any litter on the mesh screen was removed and weeds and mosses were cleared from the soil.

Monthly measurements of soil respiration and climatic conditions began in February 2009 and continued until March 2013 with a few exceptions: measurements were not taken between December 2010 and April 2011 and were unattainable during April 2012 due to site managers altering the harvest management schedules. At the same time and location of sampling soil respiration, volumetric soil moisture (0–6 cm depth) was measured using a ML2x Theta Probe and Meter HH2 (Delta T Devices, UK) as well as soil (0–7 cm depth) and air temperature measurements using a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, UK). Measurements were taken for each soil respiration chamber individually; soil moisture was measured at three points around each chamber and an average taken for each plot.

Gas Sampling and Analysis

Soil respiration (i.e. CO_2 emissions) was measured using the static chamber method described by [50], but was adapted to include the use of a pressure 'vent' (a Tedlar bag (SKC Ltd., UK) connected to the outside of the chamber using 4 mm gauge tubing [51]) designed to compensate for pressure changes within the chamber. The PVC chambers were 40 cm in diameter and 20 cm tall, with chamber design and deployment meeting the requirements outlined by de Klein and Harvey [52] with one exception: ratio of insertion depth to deployment time was only 6 cm h^{-1}. Chambers were inserted approximately 3 cm into the soil surface with exact volumes noted. This avoided severing many of the fine roots that were found very close to the soil surface, allowing total soil CO_2 flux measurements to include a more realistic estimate of belowground autotrophic respiration. Similar strategies have been recommended in different land uses by Heinemeyer et al. [53] and Mills et al. [54]. All chambers remained in the soil for the duration of the study except for at times of harvest. At times of sampling, chambers were closed with a reflective aluminium lid, which had a rubber seal around the edge to prevent leakage. In accordance with de Klein and Harvey [52], chambers were enclosed for 30 min with one 10 ml (CO_2) and one 20 ml ($^{13}CO_2$) sample taken every 10 min for a total of four time points collected at each plot. Gas samples were immediately transferred from the chamber headspace into gas-tight exetainers (Labco Ltd., UK) via a needle and syringe inserted into the self-sealing septa in the chamber lid. 10 ml exetainer gas samples were analysed for CO_2 on a Perkin-Elmer Autosystem XL Gas Chromatograph (GC) fitted with a flame ionisation detector (FID). All results were calibrated against certified gas standards (BOC, UK) [55] and converted to a total flux reported as mg CO_2-C m^{-2} h^{-1} in accordance with methods detailed in Holland et al. [56]. The majority (>85 %) of measurements were taken between the hours of 10:30 and 14:30 to represent a diurnal average [52], with some exceptions due to field logistics. Between June 2011 and February 2012, three automated static chambers (ADC BioScientific Ltd., UK) in both control and bare plots were used to measure CO_2 emissions every 3 h with an integrated Infra-Red Gas Analyser (IRGA). Although these automated chambers encountered a number of mechanical issues, their results were able to verify the assumption that daily average CO_2 emissions were comparable to the exetainer measurements taken between 10:30 and 14:30 across different growth stages of the crop. The diurnal average for the control plots was observed to be between 12:00 and 13:00 over the entire 9 months (June to February).

Isotopic Analysis and Soil Respiration Partitioning

The 20 ml samples were analysed on an Isoprime trace gas isotope mass spectrometer (TG-IRMS) and relative abundances of ^{13}C to ^{12}C in CO_2 were measured on an Isoprime isotope ratio mass spectrometer (IRMS; Isoprime UK), following its introduction to the instrument via a Trace gas preconcentration unit (Isoprime, UK). Using a gas-tight syringe, 100 μl of gas was removed from each sample vial and injected into the Tracegas pre-concentrator. The sample was then diverted through a trap filled with magnesium perchlorate to remove water, after which the CO_2 was cryogenically concentrated in glass lined cryofocussing traps immersed in liquid nitrogen. The CO_2 was then separated from residual trace gases (e.g. N_2O) on a 25 m gas chromatography capillary column filled with Poraplot Q, prior to entering the IRMS via an open split.

Reference standards of known isotopic composition (500 ppm, BOC calibrated to National Institute of Standards & Technology RM8562 (CO_2 Heavy) & RM8564 (CO_2 Biogenic)) were included after every fifteenth sample during analysis. Internal precision was better than ±0.2 ‰ for ^{13}C for the reference standards. Isotopic data are reported using delta notation relative to the international standard Vienna Pee Dee Belemnite (V-PDB).

The keeling-plot approach [57] was used to estimate the isotopic signature ($\delta^{13}C$) value from respired CO_2 for each plot for each treatment. Keeling's method shows that the integrated $^{13}CO_2$ signal produced by all components of soil respiration could be determined as the intercept of a regression of $\delta^{13}C$ versus the inverse of CO_2 concentration (ppm), where both values were collected at the same time point during chamber enclosure. Using determined $\delta^{13}C$ of sampled respiration fluxes, the following mixing model could be solved in accordance with Schnyder and Lattanzi [58]:

$$F_{C4} = \left(\delta^{13}C_R - \delta^{13}C_{C3}\right) \Big/ \left(\delta^{13}C_{C4} - \delta^{13}C_{C3}\right) \quad (1)$$

where F_{C4} is the fraction of respiration effluxed from all C4 sources (*Miscanthus*-derived), $\delta^{13}C_R$ is the isotopic signature of the gas collected from a plot, $\delta^{13}C_{C3}$ is the isotopic

signature of the C3 source (soil before *Miscanthus* was planted) and $\delta^{13}C_{C4}$ is the isotopic signature of the C4 source (*Miscanthus* biomass). The fraction of respiration effluxed from C3 sources was calculated by subtracting F_{C4} from 1.

Since the *Miscanthus* was planted 3 years before the bare plots were established, there was still a C4 component of total respiration but this did not include any CO_2 efflux from live biomass or fresh *Miscanthus* inputs (verified by no growth aboveground after trenching). As a result, C4-derived CO_2 emissions from bare plots represented the decomposition of any *Miscanthus* plant material that remained after roots were severed through trenching and aboveground litter was first removed. This allows the soil respiration from bare plots to be split into two isolated components of soil respiration: recent C input (*Miscanthus*-derived; March 2006 to January 2009) and old soil C (pre-*Miscanthus*; before March 2006). This experimental design was underpinned by the assumption that there was no C4 organic matter in the soils prior to 2006 when the *Miscanthus* was established. This assumption was verified by isotopic analysis of soil sampled to 30 cm at a directly adjacent field in 2011. Both fields had received the same management prior to 2006 and the adjacent field continued to receive the same winter wheat-oil seed rape rotation after 2006; results confirmed no measurement above −27 ‰.

Soil Sampling and Bulk ^{13}C Measurements

On the same date as the monthly gas measurements, soil samples were collected using PVC pipes (5 cm diameter) hammered into the topsoil (0–15 cm) from five locations, each of them within a 10 m radius from the static chambers. These cores were taken in March 2009 and March 2010 and then at monthly intervals from May 2011. Further, in October 2011, May 2012, October 2012 and March 2013 additional 30 cm depth cores (split into 0–15 cm and 15–30 cm layers) were taken using a 2.5 cm diameter gouge auger (Van Walt, UK). All soil collected was for destructive sampling and used for C and N determination. The soils contained less than 2 % stones by both weight and volume and therefore no correction was necessary. Routine monthly 0–15 cm cores were homogenised and freeze-dried (Alpha 1–4 LD, Martin Christ, Germany) before being gently ground by hand to pass through a 2 mm sieve. The deeper 0–30 cm cores were air-dried to constant weight at room temperature before being homogenised, ground and sieved. No differences in C or nitrogen (N) concentration were observed between the freeze-dried and air-dried samples. All visible plant matter remains (e.g. roots and leaf litter) were removed before grinding; 15 min was allocated for removing the visible plant matter from each 100 g of dry soil. Plant matter was separated into root and litter biomass (live and dead together), identified by morphological differences and quantified in both soil layers at the end of the 7th growth year for both control and bare plots (Table 1).

Small subsamples of the ground soil were taken for analysis of C and N concentration through combustion in an elemental analyser (Costech ECS 4010, Italy). C and N stocks were estimated by relation to fixed site bulk densities (1.46 g cm^{-3} for 0–15 cm and 1.53 g cm^{-3} for 15–30 cm) and the depth layer [59]. These bulk densities were taken from 15 replicates using a 4.8 cm diameter, 40 cm deep split tube sampler (Eijkelkamp Agrisearch Equipment BV, Giesbeek, Netherlands) and corrected for compression based on the depth of the hole. To ensure consistency when calculating C and N stocks, the resulting bulk density for 0–15 cm was verified against the PVC cores taken monthly. Bulk density traced throughout this period in both control and bare plots showed no significant compression or variation.

In October 2011, an adjacent field was sampled to 30 cm using the same procedure to provide a 'time-zero' for temporal analysis, as per the paired site ('space-for-time') approach. This assumed C stocks to be in equilibrium under this adjacent field—a reasonable assumption as the site had been used for the same arable crops for at least 30 years with annual tillage to 30 cm. This field had followed the same land use as the *Miscanthus* field prior to planting in 2006, was seeded with oil seed rape in 2006 and 2010, and winter wheat all other years. This arable field was tilled annually before seeding and fertilised three times each year with 35, 70 and 35 kg N ha^{-1}. Before sampling in 2011, it had recently been harvested for winter wheat before being ploughed and cultivated again. Three replicates sampled at five random locations were cored using the same split tube sampler and split into 0–15 and 15–30 cm (n = 15). The soil was then freeze-dried, sieved to 2 mm and analysed for C and N. The same procedure to remove plant matter remains from the soil samples was applied. Further, these cores were analysed for bulk density

Table 1 Estimates of oven dried root and litter plant matter (combined live and dead) from undisturbed control plots and bare plots at two soil sampling depths under a 7-year-old *Miscanthus* plantation in Lincolnshire, UK. Italicised values are totals over the top 30 cm

Treatment	Depth layer	Root matter (oven dry t ha^{-1})	Litter matter (oven dry t ha^{-1})
Control	0–15 cm	2.61 ± 0.17	4.23 ± 1.21
	15–30 cm	1.85 ± 0.58	0.26 ± 0.12
	Total	*4.46 ± 0.59*	*4.49 ± 1.20*
Bare	0–15 cm	0.21 ± 0.11	0.39 ± 0.13
	15–30 cm	0.18 ± 0.07	0.08 ± 0.03
	Total	*0.39 ± 0.16*	*0.48 ± 0.14*

and corrected for compression through coring (0–15 cm, 1.13 ± 0.17 g cm^{-3}; 15–30 cm, 1.41 ± 0.15 g cm^{-3}). C and N stocks were calculated using the field-specific bulk density values; no carbonates were detected in either field.

All soil samples were also analysed for ^{13}C concentration with an IsoPrime IRMS (Isotopx, UK) interfaced with a Euro EA 3000 elemental analyser (EuroVector, Italy). Additionally, 50 samples of *Miscanthus* biomass were collected at a number of time points between May 2011 and March 2013, dried and analysed for ^{13}C content with the same IRMS set up. These biomass samples comprised 10 measurements of recently senesced *Miscanthus* leaf litter, 10 samples of leaf matter taken from standing biomass, 10 samples of stem matter taken from standing biomass, 10 samples of belowground plant matter (from the soil cores) and 10 samples of the homogenised litter layer. As with gas samples, results were compared to reference standards and expressed as delta notation in relation to the V-PDB international standard. Although the belowground plant matter had a lower $\delta^{13}C$ signal (−12.74 ± 0.07 ‰), all other *Miscanthus* biomass was between −11.40 and −12.44 ‰. Consequently, to ensure no negative values of F_{C4} in Eq. 1, −11.40 was used as the isotopic signature for *Miscanthus* ($\delta^{13}C_{C4}$). Similarly, the ^{13}C signals of the soil samples from the paired adjacent field site were used as the isotopic signature for C3 soil ($\delta^{13}C_{C3}$) in the 0–15 and 15–30 cm layers: their values were −27.36 and −27.31, respectively. These values represented the assumed isotopic signature of soil below the *Miscanthus* field before it was planted in 2006. With these source signatures, Eq. 1 was used to calculate the relative components of C3- and C4-derived C in all samples collected from the *Miscanthus* plantation after February 2009. Relative contributions were applied to total C stocks to estimate C3 (pre-*Miscanthus*) and C4 (*Miscanthus*-derived) stocks individually. Soil cores from bare treatment plots were not analysed here.

Statistical Analyses and Calculating Rates of Change

Outliers of CO_2 measurements were determined using the assumption of normal distribution to capture 95 % of the 'real' data. That is to say, to exclude those values outside 2× standard deviation of the population mean, as per Altman and Bland [60]. Further, ^{13}C data (and therefore the isotopic partitioning for that plot) was deemed an outlier if the source was outside the reference C3 and C4 delta values (−27.36 and −11.40 ‰ for C3 and C4, respectively). All statistical analyses were performed with R version 3.0.2. [61]. User-defined growth phases of the crop were used to specify whether the *Miscanthus* was dormant (D), emerging (E) or growing (G). These each referred to 4 months of the year (November to February, March to June and July to October, respectively); the phases were found to be a significantly better predictor of CO_2 efflux than the traditional Spring, Summer, Autumn, Winter divisions. Although true crop phenology would be more accurately described by growing degree days and key climatic events (e.g. first frost), keeping these three phases equally balanced allowed more intuitive comparisons of cumulative fluxes.

Due to gaps over the 4 years of measurements, the CO_2 flux data was unbalanced and therefore comparisons between years were not made. Similarly, estimates of cumulative emissions would likely be biased towards the data present. Consequently, an average year was estimated using the data available. Fluxes for each time point were averaged across all years to determine the most realistic flux rate for an average day in a given month during the 4 years measured. Monthly averages were plotted against time and linear integration with the trapezoid rule used to calculate cumulative emissions over a given period, as described in Mancinelli et al. [62]. These cumulative emissions were calculated for each block individually to determine an average value and degree of error. The same process was repeated for the amounts of C3- and C4-derived respiration after these had been calculated according to Eq. 1.

Non-linear regression analysis was used to establish the relationship between respiration rates and soil temperature measurements. This assumed an exponential relationship and applied the 'nls' function (nonlinear least squares) as a part of the base stats package in R to estimate a Q_{10} value according to Raich and Potter [63] and Luo et al. [64]. This represented the change in respiration rate given a 10 °C rise in temperature. Although relationships were calculated using the whole dataset, a few extreme measurements of respiration skewed resulting estimates. Therefore, the temperature sensitivity was also determined using monthly averages. The results of the nls regression analysis were compared using an analysis of variance (ANOVA) to find that tests, using monthly data, were significantly ($p < 0.001$) more reliable and predicted relationships explained a larger proportion of the variance (higher r^2). The difference between the predicted relationships and a linear increase (exponent = 1) was tested using an ANOVA. While the measurements taken on any given day are unlikely to be exact averages of that month, the relationship between soil respiration and soil temperature should be reliable the many data points over the 4 years measured.

After soil C stocks had been split into C3- and C4-derived components, the rates of change were calculated assuming linear relationships. Both a simple linear function with no weighting (lm in the base stats package) and a linear mixed model (lme in the nlme package [65]) with block as a random effect were used to estimate a slope given the measured data. For estimates of the accumulation rate of *Miscanthus*-derived (C4) C, the y-axis intercept was forced to 0 because an underlying assumption of the experiment is that there was no C4 organic matter present before the plantation. For C3-derived soil C stocks, however, the y-axis intercept was allowed to vary to what the models deemed most accurate based on the data. Time points were analysed on daily time steps to ensure

the most accurate rate calculations. A pointwise 95 % confidence interval was defined around the estimated linear models to define uncertainty of the predicted relationship (and therefore calculated rates of change). Further, the significance of the predicted slope being different to 0 (i.e. no change over time) was also assessed with an ANOVA. Rates of change were calculated on a tonnes C per hectare per year basis and standard errors provided by way of uncertainty in the predicted slopes by linear regression (lm function) and linear mixed modelling (lme function).

Results

Environmental Conditions

Climatic conditions, as measured beside static chambers at the time of sampling, followed the trend of higher temperatures during the crop growth phase and showed a relatively constant soil moisture content. All data averaged by month showed July and February to have the highest and lowest soil temperatures, respectively (18.4 ± 0.5 and 1.5 ± 0.3 °C). February and April to have the highest and lowest soil moisture content, respectively (38.6 ± 0.8 and 17.3 ± 2.3 %) (Fig. S2). However, soil moisture values for April were only available for 2009 due to instrument error in 2010 and no access to the site in 2011 and 2012. Averaged by all temporal groups (e.g. season/year), no significant differences were found between control and bare plot measurements for soil temperature or soil moisture. The 4-year average soil temperatures were 11.5 ± 1.0 and 11.6 ± 1.0 °C and soil moisture measurements were 31.1 ± 1.3 and 29.5 ± 1.3 % for control and bare plots, respectively.

Isotopic Contributions to Soil Respiration within Crop Phase

Chamber soil respiration measurements were averaged by month to derive an annual scenario more representative of this plantation over the measured period. User-defined crop phases showed clear differences in emissions; for both control and bare plots total soil respiration was highest during the growth phase when soil temperatures were warmer and the crop was photosynthetically most active (Fig. 1). This was also reflected in cumulative emissions with the growth phase making up the majority of annual emissions. Emissions during the growth phase from control plots accounted for approximately three times more emissions than the maturing or dormant phases (Table 2).

When the total soil respiration was split into C3 and C4 sources, the *Miscanthus*-derived (C4) emissions consistently contributed more than 70 % of the total efflux in control plots. This was regardless of the time of year and climatic conditions, and saw no significant ($p > 0.05$) change over the 4 years in which measurements were taken (Fig. 2). When this was averaged out, the growth phase saw the largest contribution of C4 respiration (81 %) and the dormant phase the lowest (75 %).

The monthly averages within each year showed a considerable increase in C4 respiration from the maturing phase transitioning into the growth phase and the inverse was seen as the growth phase transitioned into the dormant phase. This was also reflected in cumulative fluxes (Fig. 3) where 1.41 ± 0.14 t C ha^{-1} yr^{-1} was lost from C4 sources during the average growth phase across all years. The contribution of *Miscanthus*-derived CO_2 to total soil respiration in bare

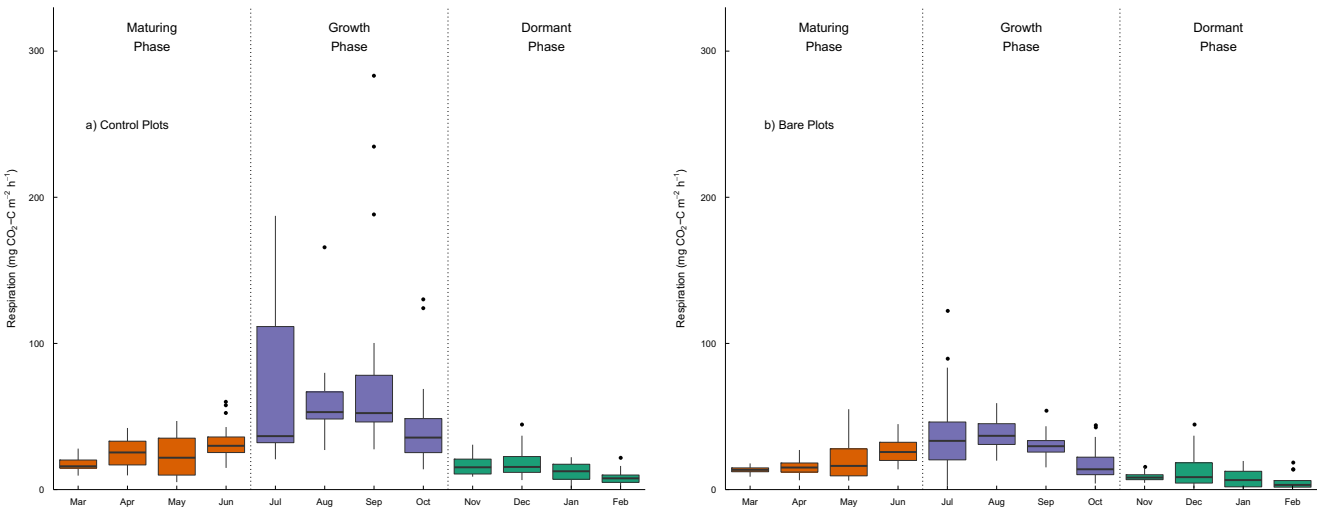

Fig. 1 Box and whisker plot to show average monthly soil respiration measured from static chambers in a commercial *Miscanthus* plantation in Lincolnshire, UK, between February 2009 and March 2013. Bare plots (**b**) have no aboveground litter additions and no live belowground biomass. Colours aid the differentiation between user-defined crop phases: *orange* describes the maturing phase (March–June); *purple*, the growth phase (July–October); *green*, the dormant phase (November–February)

Table 2 Soil respiration (±1 s.e.) from a commercial *Miscanthus* plantation in the UK. Bare plots have no fresh inputs and crop phases are split into equal time frames (Dormant, Nov–Feb; Maturing, Mar–Jun; Growth, Jul–Oct)

Treatment	Crop phase	CO_2 efflux (mg CO_2-C m^{-2} h^{-1})	Cumulative CO_2 emissions (t CO_2-C ha^{-1})	Relative C4-derived contribution (%)
Control	Dormant	14.22 ± 0.92	0.52 ± 0.05	74.93
	Maturing	24.73 ± 1.52	0.64 ± 0.03	78.04
	Growth	63.15 ± 5.63	1.84 ± 0.19	81.01
	Annual	33.99 ± 2.57	3.00 ± 0.22	79.67
Bare	Dormant	9.16 ± 0.95	0.30 ± 0.02	58.46
	Maturing	19.64 ± 1.29	0.48 ± 0.02	55.32
	Growth	30.38 ± 2.12	0.95 ± 0.04	51.89
	Annual	19.83 ± 0.77	1.73 ± 0.07	54.18

plots was significantly lower ($p < 0.01$) than in control plots, for all crop phases and annually. However the pattern was reversed, with the percentage contribution of C4-derived respiration largest during the dormant phase for bare plots and smallest during the growth phase. Cumulative emissions of C3- and C4-derived respiration were similar to each other within any phase but C4-derived emissions were notably higher when averaged over a whole year (C3, 0.70 ± 0.04 t C ha^{-1} yr^{-1}; C4, 0.83 ± 0.04 t C ha^{-1} yr^{-1}). A combination of low fluxes and outlier source values for $^{13}CO_2$ measurements during November meant not enough measurements were available to partition C3- and C4-derived soil respiration from bare plots.

Temperature Sensitivity of Soil Respiration

C3- and C4-derived soil respiration from bare and control plots was related to soil temperature to derive temperature sensitivity (Q_{10}) relationships for the whole soil respiration component. Measurements from both control and bare treatments showed that C4-derived respiration was more sensitive to changes in soil temperature than C3-derived

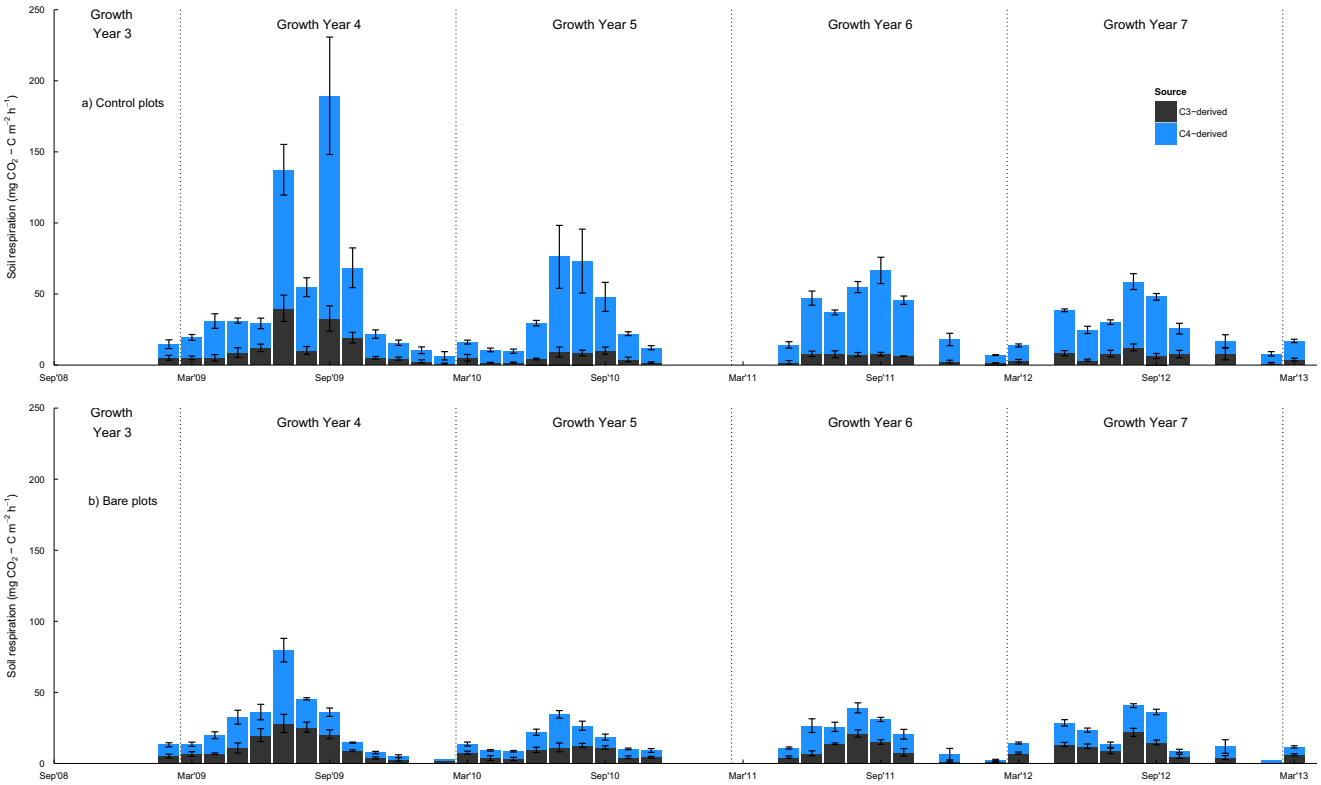

Fig. 2 Soil respiration measured below a commercial *Miscanthus* plantation split into C3 (*grey*) and C4 (*blue*) sources and averaged (±1 s.e.) for each measurement timepoint (month) between February 2009 and March 2013. *Bare plots* (**b**) have no aboveground litter additions and no live belowground biomass. Years since planting are represented by 'growth years' and consistently run from March to February of each year

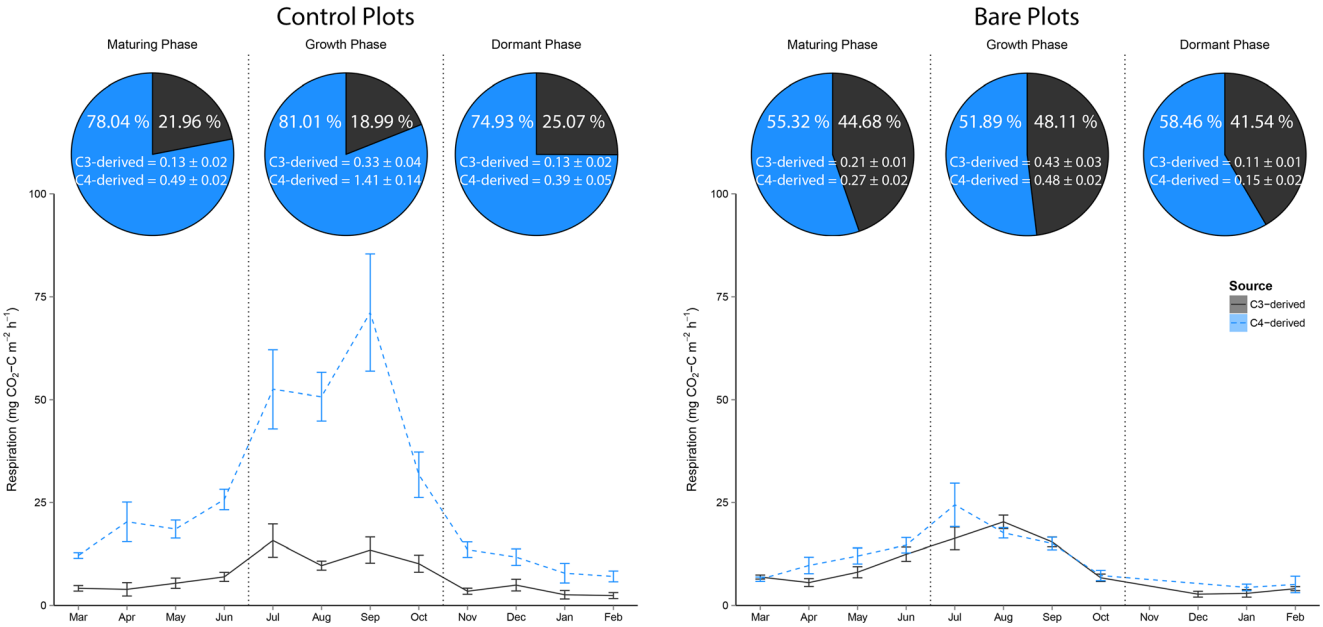

Fig. 3 Isotopic partitioning of total soil respiration to represent C3 (*grey*) and C4 (*blue*) sources of CO_2 efflux in a *Miscanthus* plantation in Lincolnshire, UK. Measurements are averaged by month (±1 s.e.) after being collected from static chambers in control (*left panel*) and bare (*right panel*) plots between February 2009 and March 2013. Pie charts show percentage contribution of C3 and C4 emissions to total soil respiration and values embedded are cumulative emissions (in t CO_2-C ha^{-1} yr^{-1}) of the C3 and C4 respiration over each of three discrete user-defined crop phases: maturing phase (March–June), growth phase (July–October) and dormant phase (November–February)

respiration (Fig. 4). In bare plots in particular the 'C4 vs temperature' relationship was relatively strong ($r^2 = 0.92$). The association between crop phase and temperature sensitivity was most notable in control plots, with growth phase measurements consistently higher than those in other crop phases. Despite differences in C3-derived respiration between measurements in control plots against bare plots, calculated temperature sensitivity was very similar.

Miscanthus-Derived Contributions to Topsoil Carbon Stocks

While total topsoil C stocks did not change significantly ($p > 0.05$) over time for any layer, March 2013 measurements (41.79 ± 2.48 and 39.53 ± 2.13 tC ha^{-1} in the top 0–15 cm and 15–30 cm soil layers, respectively) were 1.01 tC ha^{-1} higher (0–15 cm) and 0.98 tC ha^{-1} lower (15–30 cm) than in the arable field that acted as a proxy for C stocks before the *Miscanthus* was planted. However, when split into C3 and C4 components of the soil C, *Miscanthus*-derived C increased significantly ($p < 0.001$) in both the 0–15 cm and 15–30 cm layers. Although the C3 soil C saw a noticeable decline over the measurement period, large variability induced no significant change in either the 0–15 or 15–30 cm layer (Fig. S3). Both C3-derived and C4-derived soil C stocks varied considerably between monthly measurements in 2011 and 2013. However, linear relationships for C4 soil C accumulation saw a good relationship with time for both the 0–15 cm ($r^2 = 0.81$) and 15–30 cm layers ($r^2 = 0.82$) (Fig. 5; Table 3).

Rates of Change in Miscanthus-Derived Soil Carbon Stocks

The rates of change to C3-derived and C4-derived soil C stocks were estimated using both linear regression and mixed models that accounted for repeated sampling from similar areas within the plantation. Results of both tests showed the same findings, albeit with slightly different rates (Table 3). Over the whole 0–30 cm layer, C4 soil C was seen to increase by 0.86 t C ha^{-1} yr^{-1} and C3 soil C to decrease by 0.83 t C ha^{-1} yr^{-1}, though there is considerable uncertainty associated with the C3 estimated rates. Using a simple linear regression, rates to describe the total soil C in both the 0–15 cm layer and 15–30 cm layer have negative r^2 values. This suggests the regressions are less accurate at describing the relationship than a horizontal line (i.e. unchanging soil C stocks). While losses of C3-derived C were of a similar magnitude to gains in C4-derived C, rate estimates were too uncertain ($p > 0.05$) to derive reliable conclusions. High levels of confidence ($p < 0.001$) could only be placed in the rates referring to C4-derived C accumulating within either topsoil layer (Table 3).

Discussion

Miscanthus Contributions to Soil Respiration

Average C losses through soil respiration were estimated to be 3.00 ± 0.22 t C ha^{-1} yr^{-1} in control plots, with the majority

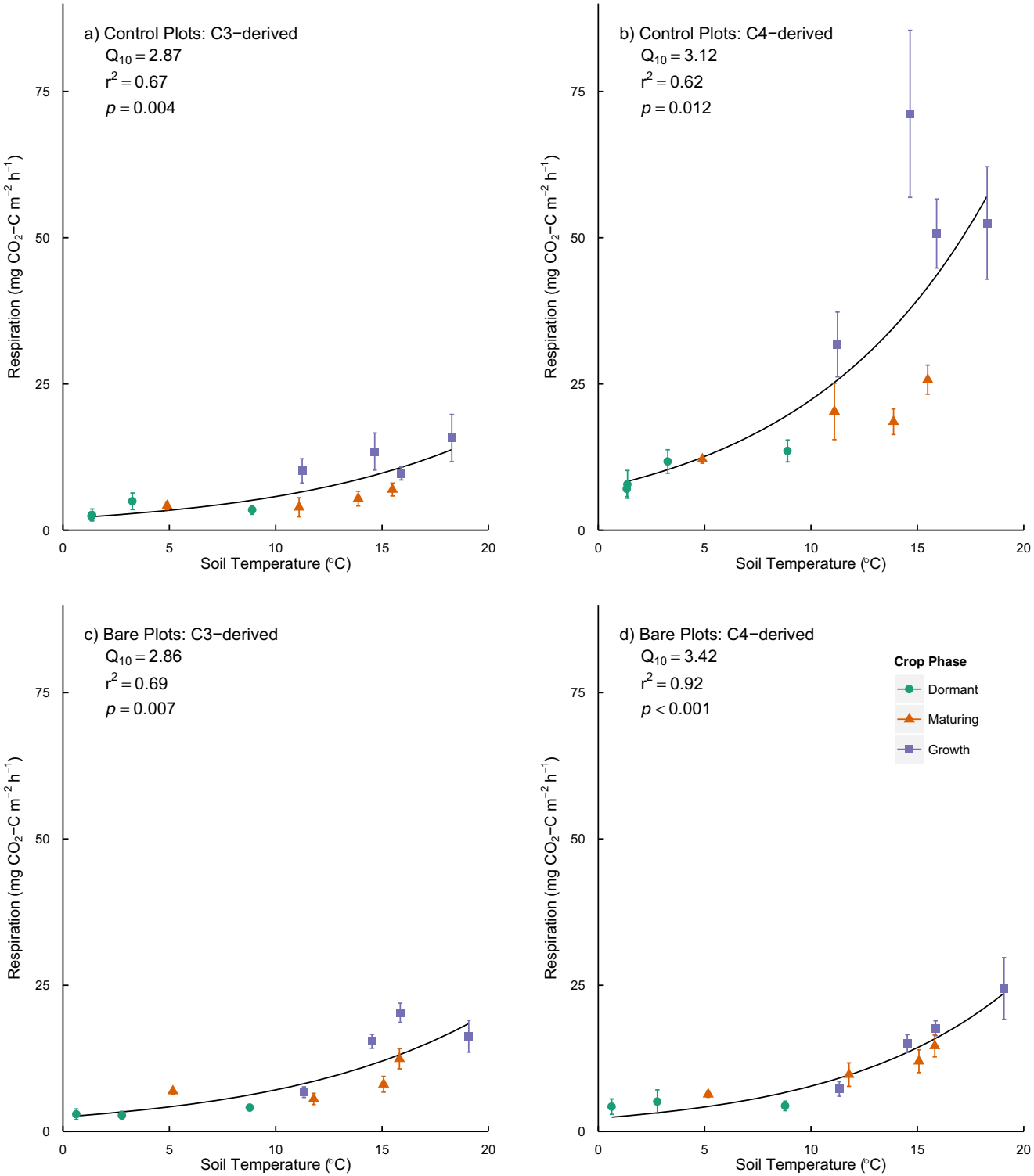

Fig. 4 Temperature sensitivity of C3-derived (**a**, **c**) and C4-derived (**b**, **d**) soil respiration for control (**a**, **b**) and bare (**c**, **d**) treatments under a *Miscanthus*-plantation in Lincolnshire, UK. Measurements were taken between February 2009 and March 2013 and averaged for each month of the year (±1 s.e.). Colours indicate the user-defined crop phase belonging to each point: *orange* describes the maturing phase (March–June); *purple*, the growth phase (July–October); *green*, the dormant phase (November–February)

(61 %) occurring between July and October, while the crop is at peak growth rates [66]. Although this was similar to measurements from bare soil (55 % from the growth phase alone), cumulative emissions were significantly lower from bare soil throughout the year (1.73 ± 0.07 t C ha^{-1} yr^{-1}). This difference is likely caused by live plant activity, and R_a in particular.

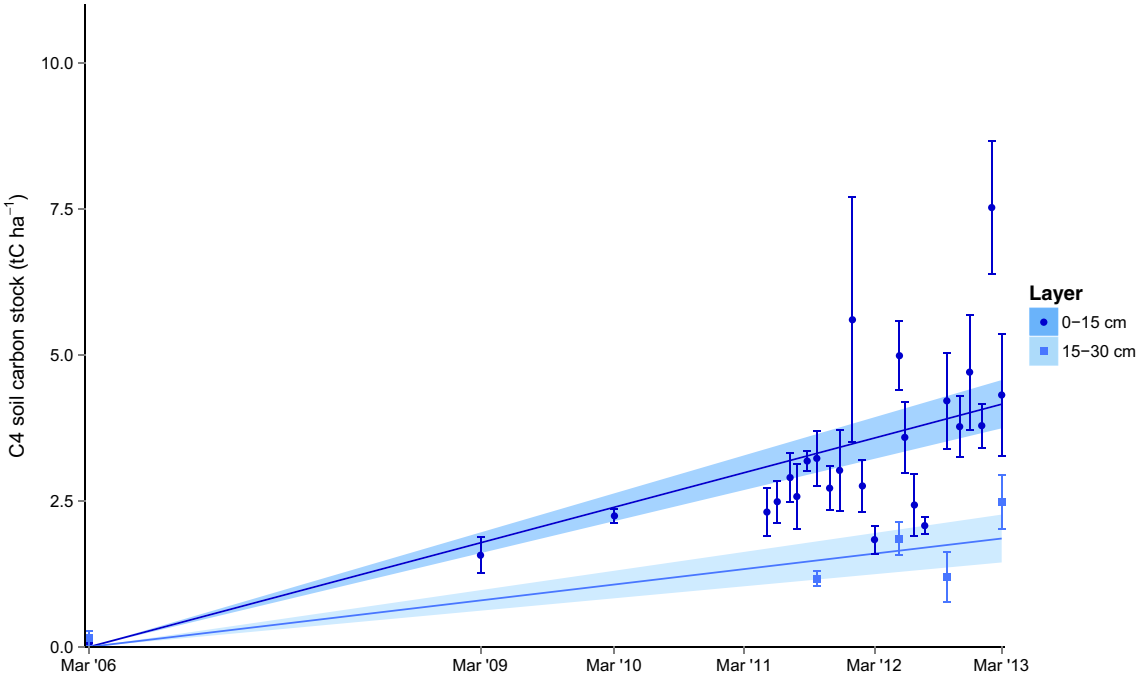

Fig. 5 Average C4-derived soil carbon stocks (±1 s.e.) below a *Miscanthus* plantation in Lincolnshire, UK, split by depth (0–15 cm, dark; 15–30 cm, light). Measurements collected between March 2009 and March 2013 with a paired-site providing a proxy for pre-establishment (March 2006) stocks; linear regressions are used to describe relationships to time with a pointwise 95 % confidence interval around the mean

Previous studies have shown that R_a often makes up a large proportion of total soil respiration and in mature forests some estimates are higher than 60 % [67]. In arable systems, however, Rochette et al. [21] showed that in a *Zea mays* plantation in Canada, R_a was a maximum of 45 % of total soil respiration during the crop's growth phase. Depending on the subtractive method used (C4 component only or totals), our study shows 48.4–50.5 % of total respiration during the whole growth phase is autotrophic (and from the associated rhizosphere). Total losses were 1.27–1.44 t C ha^{-1} yr^{-1} from R_a over a whole growing season and ~0.9 t C ha^{-1} in the growth phase alone. It is worth noting that both methods of estimating R_a agreed well for all averaged data; differences between the methods were typically within the standard errors of each estimate.

While R_a (including the associated rhizosphere) represents C that is cycled through the ecosystem very quickly [68], *Miscanthus*-derived emissions from senesced biomass or SOM is much slower. This was particularly evident as the relative contribution of C4 sources to total soil respiration in bare plots did not decline significantly over the 4 years of

Table 3 Rates of change in soil (0–30 cm) C stocks in a *Miscanthus* plantation in Lincolnshire, UK. Rates are partitioned into C3-derived and C4-derived components using simple regression analysis as well as linear mixed models where repeated measures are accounted for

Depth layer	Soil partition	Model type	Rate of change (tC ha^{-1} yr^{-1})	r^2	F-statistic	p value
0–15 cm	Total	Linear regression	0.235 ± 0.310	−0.00	0.6	0.450
		LME	0.242 ± 0.304	0.01	0.6	0.428
	C3-Carbon	Linear regression	−0.441 ± 0.254	0.02	3.0	0.086
		LME	−0.433 ± 0.248	0.03	3.0	0.085
	C4-Carbon	Linear regression	0.593 ± 0.030	0.81	402.7	<0.001
		LME	0.593 ± 0.031	0.24	357.6	<0.001
15–30 cm	Total	Linear regression	−0.136 ± 0.369	−0.05	0.1	0.717
		LME	−0.134 ± 0.368	0.01	0.1	0.721
	C3-Carbon	Linear regression	−0.385 ± 0.373	0.00	1.1	0.316
		LME	−0.385 ± 0.373	0.05	1.1	0.320
	C4-Carbon	Linear regression	0.265 ± 0.028	0.82	89.9	<0.001
		LME	0.263 ± 0.032	0.54	68.4	<0.001

measurements, despite bare and control plots starting from very similar conditions and only 3 years of inputs prior to root/litter exclusion. Further, the C4 component of bare soil respiration was, on average, higher than the C3 component in all phases and annually. With estimated losses of 0.83 ± 0.04 t C ha^{-1} yr^{-1} in C4 soil respiration between 2009 and 2013, we can assume that, during the first 3 years after establishing the plantation, at least 1.11 t C ha^{-1} yr^{-1} was added to the entire soil profile. Though it is unclear how much was added from severed roots as a result of trenching and what was added through natural root turnover before trenching. As time passes, the amount of labile C4 organic matter will decrease as it is decomposed and it is likely that more stable C compounds will remain with slower decomposition rates [69]. Without fresh additions of C3 biomass, the same will be true for C3 soil C stocks.

Temperature Sensitivity of Old vs New SOM Decomposition

The relationship between soil temperature and total respiration from undisturbed soils cannot be accurately described using simple regression analysis due to the influence of R_a that varies with plant activity. In situ SOM decomposition, however, agrees well with the assumption of an exponential relationship. Our results showed that decomposition of old SOM (C3) increased considerably as temperature rose; each 10 °C increase saw CO_2 emissions from C3 sources increase by ~286 % (Q_{10} = 2.86). Although relatively high, this figure is in line with many other arable systems [70, 71] and research suggests that site-specific values are more closely linked to plant phenology and climatic conditions than to land use and management practices [72, 73]. Interestingly, the C3-derived respiration rates were similar in both control and bare plots. This suggests that inputs of *Miscanthus* plant matter, and effects of live belowground biomass, do not stimulate a noteworthy increase in microbial activity acting on C3 SOM decomposition. This does not mean the microbial community is the same as the adjacent arable land, just that the activity is similar. Fierer et al. [74] observed that the temperature sensitivity of SOM is more accurately related to its quality than to the age or extent of decomposition. While the temperature sensitivity of C3 SOM decomposition was not seen to change when fresh inputs were removed, the cumulative emissions from C3-sources were lower in control plots (0.58 vs 0.70 t C ha^{-1} yr^{-1}). This suppression of emissions may be due to there being more labile C4-C available for microbial decomposition, resulting in less activity on the C3-C [74].

Decomposition of recent (2006–2009) additions to SOM appeared to be more sensitive to temperature than the decomposition of old SOM. The best fit exponential model explained a large proportion of the variation for measured monthly C4-derived respiration data from bare plots (r^2 = 0.92) and indicated that a 10 °C rise in soil temperature would increase CO_2 losses by 342 % (Q_{10} = 3.42). The increased temperature sensitivity of recent additions to SOM suggests that for this pool, the lability of its C is less limiting than that of older SOM C [35, 74, 75]. This has important consequences for the mean residence time (MRT) of C after its sequestration in soils. Although this study does not differentiate emissions from different soil fractions, it is important to note that if fresh additions of *Miscanthus* biomass are likely to be lost from the soil within a few years of addition then its potential climate change mitigation by acting as a C sink is not a long-term solution. Previous studies have shown that the MRT of C sequestered in soils can be strongly linked to climatic conditions [76], clay content [77] and fresh inputs [20]. Our results suggest that recent additions are more sensitive to temperature than older additions but due to the lack of data we could not create reliable estimates of temperature sensitivity for each year individually. Annual Q_{10} estimates could provide an indication of how long the recent C additions remain more sensitive than older SOM C. However, the use of the Q_{10} approach must be treated with caution as it typically underestimates the sensitivity at high temperatures [78] and its relationship to the recalcitrance of decomposed substrate is currently unclear [79–82]. Simultaneous measurements of soil C stability and annual Q_{10} estimates are needed to help resolve this relationship, and therefore determine how soil respiration is changed as the lability of the new SOM gets closer to that of the older SOM. Further, more recent studies also suggest that specific microbial processes, including growth and priming effects should also be considered to further our understanding of the temperature sensitivity of SOM decomposition [83, 84].

Changes in Topsoil Carbon Stocks

At the same site, a twinned study noted that total topsoil (0–30 cm) C stocks did not change significantly over the first 7 years of growth [7]. However, we found *Miscanthus*-derived (C4) SOM to accumulate steadily over the same period, at a rate of 0.86 t C ha^{-1} yr^{-1} (0.59 in the 0–15 cm layer and 0.27 in the 15–30 cm layer). Despite considerable variation between time points, the confidence in these calculated rates of accumulation being significantly above 0 was high, particularly for the 0–15 cm layer. After 7 years of growth, in March 2013, the C4 component accounted for 10.3 % of total 0–15 cm C stocks and 8.4 % in the whole 0–30 cm. This compares well with Hansen et al. [16] who noted 13 % of 0–20 cm soil C was C4-derived in a 9-year-old *Miscanthus* plantation. Due to the measured variability in C3-derived soil C, any losses since measurements began in 2009 were negligible (i.e. not significantly different to a 'no change' scenario). This variation was however sufficient to suppress the significant accumulation of *Miscanthus*-derived C in topsoil (0–30 cm) stocks (6.80 t C ha^{-1} after 7 years) and suggest no significant change in the overall C stock. Consequently, a rate of C3-C loss similar to

that estimated in this study (~0.83 t C ha^{-1} yr^{-1}) could be expected. The variation in C3-derived soil C measurements could not be explained by those variables measured, but spatial heterogeneity is likely to account for much of the difference noted between and within time points [85]. Additionally, only three data points represent the change over the first 5 years and therefore their influence in regression analysis over time is high in comparison to the bulk of measurements that occurred over the last 2 years. That said, this approach is the best available to provide estimates of rates, and our findings compliment those in Robertson et al. [7] by helping to explain unchanging total soil C stocks: new SOM is likely to be displacing old SOM, resulting in a net balance of total topsoil C.

Evidence for Priming of SOM

A comparison of partitioned respiration between treatments showed no significant difference in C3-derived emissions; on average, CO_2 respired from C3 sources under control plots was 0.12 t C ha^{-1} yr^{-1} lower than under bare plots. This suggests that decomposition of C3 SOM is slower under control plots where fresh inputs and live roots are present. This indicates no apparent priming effect as a result of fresh *Miscanthus* inputs. Although soil C stocks from bare plots were not studied here, the lack of increased respiration is good evidence that decomposition of old (>8 years) SOM was not accelerated over the measurement period. When measuring the conversion of C3 grassland to *Miscanthus*, Zatta et al. [41] noted that grassland SOM decreased more in the presence of higher belowground biomass, hypothesising that these losses were due to rhizosphere priming. While their study used trial sites of 25 m^2 and achieved higher yields, the soil type, climatic conditions, plantation age and topsoil C stocks were similar to those reported here. The differences between these two studies may be due to the quantity and quality of the C3 SOM; generally perennial ryegrass (the prior land use in Zatta et al. [41]) has more fine root biomass in topsoils [86], lower C:N [87] and lower lignin content [88] than winter wheat (the prior land use in our study) [89, 90]. This may suggest that while priming does act on the grassland SOM, it does not on the winter wheat SOM.

In our study, rhizosphere priming is more likely to be detectable than priming effects as a result of C fertilisation through organic matter inputs. Further, undisturbed soils, like those below the studied *Miscanthus* plantation, have less oxygen available for decomposition and therefore microbial activity is stimulated more by the influence of root biomass [91]. Consequently, emphasis for this discussion is placed on the influence of the *Miscanthus* root activity. Low yields are a good indicator that root biomass at our site is lower than in more productive plantations. Therefore, the amount of rhizospheric deposition will be lower, thus influencing any rhizosphere priming. Additionally, Hromádko et al. [92] observed *Miscanthus* root exudation and rhizodeposition to be low, even in comparison to similar C4 crops like maize [93, 94]. Kaňová et al. [95] also observed that rhizosphere respiration under *Miscanthus* was limited by nitrogen, potassium and calcium, further reinforcing the idea that a lack of priming in our study may, in part, be due to the high C:N of *Miscanthus* biomass. Other studies have also suggested a mechanistic links between N-availability and priming SOM decomposition [96, 97]. Rhizospheric priming in particular is intrinsically linked to N-mineralisation, with the extent of live root biomass being strongly linked to the microbial activity associated with decomposition. Indeed, Zhu et al. [98] found that the presence of roots increased microbial biomass C by up to 28 %, leading to positive soil C priming of 45–79 % and increased N-mineralisation of 10–52 %. This provides further evidence that low N availability may limit soil C priming at our site. A lack of priming, however, removes one possible explanation for the unchanging topsoil C stocks and indicates that a simple substitution of old soil C for new additions may be more likely.

This research highlights the importance of partitioning new and old C dynamics in order to best quantify the relationships between driving variables and the key fluxes that define a site's long-term environmental sustainability. Through combined input manipulation treatments and isotopic partitioning we saw that soil respiration was dominated by emissions from *Miscanthus*-derived sources; belowground R_a accounts for ~50 % of the annual emissions and heterotrophic respiration of recently added (≤7 years) SOM accounts for approximately 30 %. Further, there was no apparent priming effect through rhizodeposition or aboveground litter inputs. In fact, less emissions from decomposition of older (>7 years) SOM were seen from those plots with fresh inputs. In addition, old SOM was seen to be less sensitive to temperature than newer SOM. Despite substantial emissions of around 3 t C ha^{-1} yr^{-1}, topsoil (0–30 cm) C stocks did not change significantly during the 7 years after the *Miscanthus* was planted. The accumulation of fresh *Miscanthus* inputs was seen to displace losses from old SOM. While the unchanging topsoil C stocks contrast substantial gains observed in similar *Miscanthus* plantations across Europe, the accumulation of *Miscanthus*-derived C is in excess of the average reported in the Poeplau and Don [8] multisite evaluation. From this study, it is unclear why decomposition of old SOM exceeds that observed elsewhere but, importantly, if the sequestration of *Miscanthus*-C is in stable forms, the loss of the more labile SOM is of less concern within the context of sustainability assessments. With *Miscanthus* and many other bioenergy crops, we have a prime opportunity to help mitigate climate change through displacing fossil fuel energy generation while removing CO_2 from the atmosphere and storing it in soils. However, to correctly assess the impacts of these bioenergy crops, we must first establish what the key drivers of sequestration are, as well as the realised impacts of bioenergy crops on this sequestration.

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References

1. Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42
2. Stern N., 2006 Stern Review: The economics of climate change. Vol. 30. London: HM treasury.
3. IPCC (2014) Climate change 2014: impacts, adaptation, and vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (eds) Summary for policymakers. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. Pp. 1–Pp.32
4. Brandão M, Milà i Canals L, Clift R (2011) Soil organic C changes in the cultivation of energy crops: implications for GHG balances and soil quality for use in LCA. Biomass Bioenergy 35(6):2323–2336
5. Hamelin L, Jørgensen U, Petersen BM, Olesen JE, Wenzel H (2012) Modelling the C and nitrogen balances of direct land use changes from energy crops in Denmark: a consequential life cycle inventory. GCB Bioenergy 4(6):889–907
6. Hillier J, Whittaker C, Dailey G, Aylott M, Casella E, Richter GM, Riche A, Murphy R, Taylor G, Smith P (2009) Greenhouse gas emissions from four bioenergy crops in England and Wales: integrating spatial estimates of yield and soil C balance in life cycle analyses. Glob Chang Biol Bioenergy 1:267–281
7. Robertson A.D., Whitaker J., Morrison R., Davies C.A., Smith P., McNamara N.P., 2016. A *Miscanthus* plantation can be carbon neutral without net sequestration in soils. *Global Change Biology Bioenergy*, in review.
8. Poeplau C, Don A (2014) Soil C changes under *Miscanthus* driven by C4 accumulation and C3 decompostion—toward a default sequestration function. Glob Chang Biol Bioenergy 6(4):327–338
9. Howes P., Barker N., Higham I., O'Brien S., Talvitie M., Bates J., Adams M., Jones H., Dumbleton F., 2002. Review of Power Production from Renewable and Related Sources. R & D Technical Report P 4–097/TR, Environment Agency, Bristol, UK.
10. Lewandowski I, Clifton-Brown JC, Scurlock JMO, Huisman W (2000) *Miscanthus*: European experience with a novel energy crop. Biomass Bioenergy 19(4):209–227
11. Christian DG, Riche AB, Yates NE (2008) Growth, yield and mineral content of *Miscanthus x giganteus* grown as a biofuel for 14 successive harvests. Ind Crop Prod 28(3):320–327
12. Clifton-Brown JC, Stampfl PF, Jones MB (2004) *Miscanthus* biomass production for energy in Europe and its potential contribution to decreasing fossil fuel C emissions. Glob Chang Biol 10:509–518
13. Zimmermann J, Dauber J, Jones MB (2012) Soil C sequestration during the establishment phase of *Miscanthus* x *giganteus*: a regional-scale study on commercial farms using ^{13}C natural abundance. Glob Chang Biol Bioenergy 4:453–461
14. Robertson AD, Davies CA, Smith P, Dondini M, McNamara NP (2015) Modelling the C cycle of *Miscanthus* plantations: existing models and the potential for their improvement. Glob Chang Biol Bioenergy 7(3):405–421
15. Dondini M, Hastings A, Saiz G, Jones MB, Smith P (2009) The potential of *Miscanthus* to sequester C in soils: comparing field measurements in Carlow, Ireland to model predictions. Glob Chang Biol Bioenergy 1:413–425
16. Hansen EM, Christensen BT, Jensen LS, Kristensen K (2004) C sequestration in soil beneath long-term *Miscanthus* plantations as determined by ^{13}C abundance. Biomass Bioenergy 26:97–105
17. Amougou N, Bertrand I, Cadoux S, Recous S (2012) *Miscanthus* × giganteus leaf senescence, decomposition and C and N inputs to soil. GCB Bioenergy 4:698–707
18. Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498
19. Fontaine S, Bardoux G, Abbadie L, Mariotti A (2004) C input to soil may decrease soil C content. Ecol Lett 7(4):314–320
20. Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic C in deep soil layers controlled by fresh C supply. Nature 450:277–280
21. Rochette P, Flanagan LB, Gregorich EG (1999) Separating soil respiration into plant and soil components using analyses of the natural abundance of C-13. Soil Sci Soc Am J 63:1207–1213
22. Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA (2003) The application and interpretation of keeling plots in terrestrial C cycle research. Glob Biogeochem Cycles 17:1022
23. Balesdent J, Mariotti A, Guillet B (1987) Natural 13C abundance as a tracer for studies of soil organic matter dynamics. Soil Biol Biochem 19:25–30
24. Boyero L, Cardinale BJ, Bastian M, Pearson RG (2014) Biotic vs. abiotic control of decomposition: a comparison of the effects of simulated extinctions and changes in temperature. PLoS One 9:e87426
25. Hartley IP, Ineson P (2008) Substrate quality and the temperature sensitivity of soil organic matter decomposition. Soil Biol Biochem 40:1567–1574
26. Davidson EA, Janssens IA (2006) Temperature sensitivity of soil C decomposition and feedbacks to climate change. Nature 440:165–173
27. Lal R (2004) Soil C sequestration to mitigate climate change. Geoderma 123:1–22
28. Jones RJ, Hiederer R, Rusco E, Montanarella L (2005a) Estimating organic C in the soils of Europe for policy support. Eur J Soil Sci 56:655–671
29. Cox PM, Betts RA, Jones CD, Spall SA, Totterdell IJ (2000) Acceleration of global warming due to C-cycle feedbacks in a coupled climate model. Nature 408:184–187
30. Burke IC, Kaye JP, Bird SP, Hall SA, McCulley RL, Sommerville GL (2003) Evaluating and testing models of terrestrial biogeochemistry: the role of temperature in controlling decomposition. In: Canham CD, Cole JJ, Lauenroth WK (eds) Models in ecosystem science. Princeton University Press, Princeton, NJ, Pp, pp. 225–253
31. Jones C, McConnell C, Coleman K, Cox P, Falloon P, Jenkinson D, Powlson D (2005b) Global climate change and soil C stocks; predictions from two contrasting models for the turnover of organic C in soil. Glob Chang Biol 11:154–166
32. Ågren GI (2000) Temperature dependence of old soil organic matter. Comments on the paper by Liski *et al*., 1999, in: *AMBIO: A Journal of the Human*. Environment 29:55–55
33. Giardina CP, Ryan MG (2000) Evidence that decomposition rates of organic C in mineral soil do not vary with temperature. Nature 404:858–861

34. Reichstein M, Kätterer T, Andren O, Ciais P, Schulze ED, Cramer W, Papale D, Valentini R (2005) Temperature sensitivity of decomposition in relation to soil organic matter pools: critique and outlook. Biogeosciences 2:317–321
35. Vanhala P, Karhu K, Tuomi M, Sonninen E, Jungner H, Fritze H, Liski J (2007) Old soil C is more temperature sensitive than the young in an agricultural field. Soil Biol Biochem 39:2967–2970
36. Fang C, Smith P, Moncrieff JB, Smith JU (2005) Similar response of labile and resistant soil organic matter pools to changes in temperature. Nature 433:57–59
37. Karhu K, Auffret MD, Dungait JA, Hopkins DW, Prosser JI, Singh BK, Subke J-A, Wookey PA, Ågren GI, Sebastià M-T, Gouriveau F, Bergkvist G, Meir P, Nottingham AT, Salinas N, Hartley IP (2014) Temperature sensitivity of soil respiration rates enhanced by microbial community response. Nature 513:81–84
38. Tjoelker MG, Oleksyn J, Reich PB (2001) Modelling respiration of vegetation: evidence for a general temperature-dependent Q_{10}. Glob Chang Biol 7(2):223–230
39. Davidson EA, Janssens IA, Luo Y (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q10. Glob Chang Biol 12:154–164
40. Buchmann N (2000) Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. Soil Biol Biochem 32:1625–1635
41. Zatta A, Clifton-Brown J, Robson P, Hastings A, Monti A (2014) Land use change from C3 grassland to C4 *Miscanthus*: effects on soil C content and estimated mitigation benefit after six years. Glob Chang Biol Bioenergy 6(4):360–370
42. Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371
43. Blagodatskaya E, Yuyukina T, Blagodatsky S, Kuzyakov Y (2011) Three-source-partitioning of microbial biomass and of CO_2 efflux from soil to evaluate mechanisms of priming effects. Soil Biol Biochem 43:778–786
44. Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45(2):115–131
45. Kuzyakov Y, Bol R (2006) Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol Biochem 38:747–758
46. Zimmermann J, Styles D, Hastings A, Dauber J, Jones MB (2014) Assessing the impact of within crop heterogeneity ('patchiness') in young *Miscanthus* × *giganteus* fields on economic feasibility and soil carbon sequestration. GCB Bioenergy 6:566–576
47. Neukirchen D, Himken M, Lammel J, Czypionka-Krause U, Olfs HW (1999) Spatial and temporal distribution of the root system and root nutrient content of an established *Miscanthus* crop. Eur J Agron 11(3):301–309
48. Unger PW, Kaspar TC (1994) Soil compaction and root growth: a review. Agron J 86(5):759–766
49. Monti A, Zatta A (2009) Root distribution and soil moisture retrieval in perennial and annual energy crops in Northern Italy. Agric Ecosyst Environ 132(3):252–259
50. Livingston GP, Hutchinson GL (1995) Enclosure-based measurement of trace gas exchange: applications and sources of error. In: Matson PA, Harris RC (eds) Biogenic trace gases: measuring emissions from soil and water. Marston Lindsey Ross International Ltd., Oxford, pp. 14–51
51. Nakano T, Inoue GEN, Fukuda M (2004) Methane consumption and soil respiration by a birch forest soil in West Siberia. Tellus B 56(3):223–229
52. de Klein, C.A.M., Harvey M.J., 2015. Nitrous oxide chamber methodology guidelines – Version 1.1. *Ministry for Primary Industries*, pp. 146. Found at: http://globalresearchalliance.org/wp-content/uploads/2015/11/Chamber_Methodology_Guidelines_Final-V1.1-2015.pdf. [Last accessed 24 February 2016].
53. Heinemeyer A, Di Bene C, Lloyd AR, Tortorella D, Baxter R, Huntley B, Gelsomino A, Ineson P (2011) Soil respiration: implications of the plant-soil continuum and respiration chamber collar-insertion depth on measurement and modelling of soil CO_2 efflux rates in three ecosystems. Eur J Soil Sci 62:82–94
54. Mills R, Glanville H, McGovern S, Emmett B, Jones DL (2011) Soil respiration across three contrasting ecosystem types: comparison of two portable IRGA systems. J Plant Nutr Soil Sci 174(4): 532–535
55. Case SDC, McNamara NP, Reay DS, Whitaker J (2014) Can biochar reduce soil greenhouse gas emissions from a *Miscanthus* bioenergy crop? Glob Chang Biol Bioenergy 6:76–89
56. Holland EA, Robertson GP, Greenberg J, Groffman PM, Boone RD, Gosz JR (1999) Standard soil methods for long-term ecological research. In: GP R, DC C, CS B, Sollins P (eds) Soil CO_2, N_2O, and CH_4 exchange. Oxford University Press, Oxford, pp. pp 185–pp 201
57. Keeling CD (1958) The concentration and isotopic abundances of atmospheric C dioxide in rural areas. Geochim Cosmochim Acta 13(4):322–334
58. Schnyder H, Lattanzi FA (2005) Partitioning respiration of C3-C4 mixed communities using the natural abundance 13C approach-testing assumptions in a controlled environment. Plant Biol 7(6): 592–600
59. Guo LB, Gifford RM (2002) Soil carbon stocks and land use change: a meta-analysis. Glob Chang Biol 8(4):345–360
60. Altman DG, Bland JM (1995) Statistics notes: the normal distribution. Br Med J 310:298
61. R Core Team (2014). R Foundation for Statistical Computing, Vienna, Austria.
62. Mancinelli R, Campiglia E, Di Tizio A, Marinari S (2010) Soil C dioxide emission and C content as affected by conventional and organic cropping systems in Mediterranean environment. Appl Soil Ecol 46:64–72
63. Raich JW, Potter CS (1995) Global patterns of C dioxide emissions from soils. Glob Biogeochem Cycles 9:23–36
64. Luo Y, Wan S, Hui D, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. Nature 413:622–625
65. Pinheiro J., Bates D., DebRoy S., Sarkar D., R Development Core Team, 2013. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–113.
66. Beale CV, Bint DA, Long SP (1996) Leaf photosynthesis in the C4-grass *Miscanthus* x *giganteus*, growing in the cool temperate climate of southern England. J Exp Bot 47:267–273
67. Olsson P, Linder S, Giesler R, Högberg P (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Glob Chang Biol 11:1745–1753
68. Ryan MG, Law BE (2005) Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73:3–27
69. Blair GJ, Lefroy RD, Lisle L (1995) Soil C fractions based on their degree of oxidation, and the development of a C management index for agricultural systems. Crop Pasture Sci 46(7):1459–1466
70. Kutsch WL, Kappen L (1997) Aspects of C and nitrogen cycling in soils of the Bornhöved Lake district II. Modelling the influence of temperature increase on soil respiration and organic C content in arable soils under different managements. Biogeochemistry 39(2):207–224
71. Zheng ZM, Yu GR, Fu YL, Wang YS, Sun XM, Wang YH (2009) Temperature sensitivity of soil respiration is affected by prevailing climatic conditions and soil organic C content: a trans-China based case study. Soil Biol Biochem 41:1531–1540
72. Janssens IA, Carrara A, Ceulemans R (2004) Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity. Glob Chang Biol 10(2):161–169
73. von Lützow M, Kögel-Knabner I (2009) Temperature sensitivity of soil organic matter decomposition—what do we know? Biol Fertil Soils 46:1–15

74. Fierer N, Craine JM, McLauchlan K, Schimel JP (2005) Litter quality and the temperature sensitivity of decomposition. Ecology 86:320–326
75. Cadisch G., Giller K.E., 1997 Driven by nature: plant litter quality and decomposition. CAB international.
76. Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil C turnover to warming. Nature 433:298–301
77. Wattel-Koekkoek EJW, Buurman P, Van Der Plicht J, Wattel E, Van Breemen N (2003) Mean residence time of soil organic matter associated with kaolinite and smectite. Eur J Soil Sci 54(2):269–278
78. Kirschbaum MU (1995) The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biol Biochem 27(6):753–760
79. Liski J, Ilvesniemi H, Mäkelä A, Westman CJ (1999) CO_2 emissions from soil in response to climatic warming are overestimated: the decomposition of old soil organic matter is tolerant of temperature. Ambio 28:171–174
80. Conen F, Karhu K, Leifeld J, Seth B, Vanhala P, Liski J, Alewell C (2008) Temperature sensitivity of young and old soil carbon—same soil, slight differences in ^{13}C natural abundance method, inconsistent results. Soil Biol Biochem 40(10):2703–2705
81. Craine JM, Fierer N, McLauchlan KK (2010) Widespread coupling between the rate and temperature sensitivity of organic matter decay. Nat Geosci 3(12):854–857
82. Hamdi S, Moyano F, Sall S, Bernoux M, Chevallier T (2013) Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. Soil Biol Biochem 58:115–126
83. Zhu B, Cheng W (2011) Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Glob Chang Biol 17(6):2172–2183
84. Thiessen S, Gleixner G, Wutzler T, Reichstein M (2013) Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass–an incubation study. Soil Biol Biochem 57:739–748
85. Cambardella CA, Moorman TB, Parkin TB, Karlen DL, Novak JM, Turco RF, Konopka AE (1994) Field-scale variability of soil properties in Central Iowa soils. Soil Sci Soc Am J 58(5):1501–1511
86. Schenk U, Manderscheid R, Hugen J, Weigel HJ (1995) Effects of CO_2 enrichment and intraspecific competition on biomass partitioning, nitrogen content and microbial biomass C in soil of perennial ryegrass and white clover. J Exp Bot 46:987–993
87. Schnyder H, de Visser R (1999) Fluxes of reserve-derived and currently assimilated C and nitrogen in perennial ryegrass recovering from defoliation. The regrowing tiller and its component functionally distinct zones. Plant Physiol 119:1423–1436
88. Smith KF, Simpson RJ, Oram RN, Lowe KF, Kelly KB, Evans PM, Humphreys MO (1998) Seasonal variation in the herbage yield and nutritive value of perennial ryegrass (*Lolium perenne* L.) cultivars with high or normal herbage water-soluble carbohydrate concentrations grown in three contrasting Australian dairy environments. Anim Prod Sci 38:821–830
89. van Noordwijk M, Brouwer G, Koning H, Miejboom FW, Grzebisz W (1994) Production and decay of structural root material of winter wheat and sugar beet in conventional and integrated cropping systems. Agric Ecosyst Environ 51:99–113
90. Kriauciuniene Z, Velicka R, Raudonius S, Rimkeviciene M (2008) Changes of lignin concentration and C: N in oilseed rape, wheat and clover residues during their decomposition in the soil. Agron Res 6: 489–498
91. Parr JF, Reuszer HW (1959) Organic matter decomposition as influenced by oxygen level and method of application to soil. Soil Sci Soc Am J 23(3):214–216
92. Hromadko L., Vranova V., Techer D., Laval-Gilly P., Rejsek K., Formanek P., Falla J., 2010. Composition of root exudates of *Miscanthus x Giganteus* Greef et Deu. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis (Czech Republic).
93. Kraffczyk I, Trolldenier G, Beringer H (1984) Soluble root exudates of maize: influence of potassium supply and rhizosphere microorganisms. Soil Biol Biochem 16(4):315–322
94. Zhang J, Zhang X, Liang J (1995) Exudation rate and hydraulic conductivity of maize roots are enhanced by soil drying and abscisic acid treatment. New Phytol 131(3):329–336
95. Kanová H., Carre J., Vránová V., Rejsek K., Formánek P., 2010. Organic compounds in root exudates of *Miscanthus x giganteus* Greef et Deu and limitation of microorganisms in its rhizosphere by nutrients. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis (Czech Republic).
96. Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature 419(6910):915–917
97. Chen B, Liu E, Tian Q, Yan C, Zhang Y (2014) Soil nitrogen dynamics and crop residues. A review. Agron Sustain Dev 34(2):429–442
98. Zhu B, Gutknecht JL, Herman DJ, Keck DC, Firestone MK, Cheng W (2014) Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biol Biochem 76:183–192