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Land use change from C3 grassland to C4 *Miscanthus*: effects on soil carbon content and estimated mitigation benefit after six years

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Abstract

To date, most *Miscanthus* trials and commercial fields have been planted on arable land. Energy crops will need to be grown more on lower grade lands unsuitable for arable crops. Grasslands represent a major land resource for energy crops. In grasslands, where soil organic carbon (SOC) levels can be high, there have been concerns that the carbon mitigation benefits of bioenergy from *Miscanthus* could be offset by losses in SOC associated with land use change. At a site in Wales (UK), we quantified the relatively short-term impacts (6 years) of four novel *Miscanthus* hybrids and *Miscanthus* × *giganteus* on SOC in improved grassland. After 6 years, using stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$), the amount of *Miscanthus* derived C (C4) in total SOC was considerable (ca. 12%) and positively correlated to belowground biomass of different hybrids. Nevertheless, significant changes in SOC stocks (0–30 cm) were not detected as C4 *Miscanthus* carbon replaced the initial C3 grassland carbon; however, initial SOC decreased more in the presence of higher belowground biomass. We ascribed this apparently contradictory result to the rhizosphere priming effect triggered by easily available C sources. Observed changes in SOC partitioning were modelled using the RothC soil carbon turnover model and projected for 20 years showing that there is no significant change in SOC throughout the anticipated life of a *Miscanthus* crop. We interpret our observations to mean that the new labile C from *Miscanthus* has replaced the labile C from the grassland and, therefore, planting *Miscanthus* causes an insignificant change in soil organic carbon. The overall C mitigation benefit is therefore not decreased by depletion of soil C and is due to substitution of fossil fuel by the aboveground biomass, in this instance 73–108 Mg C ha⁻¹ for the lowest and highest yielding hybrids, respectively, after 6 years.

Keywords: bioenergy, grassland, *Miscanthus*, priming effect, roots, SOC, Stable carbon isotope

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Introduction

The European renewable energy directive 2009/28/EC (E.C., 2009) provides a legislative framework for reducing GHG emissions by 20%, while achieving a 20% share of energy from renewable sources by 2020. Energy crops, particularly perennial grasses, can contribute to both targets by replacing fossil fuel energy sources, as well as increasing soil organic carbon (SOC) sequestration, i.e. the long-term storage of carbon in soil. It has been estimated that in the next 50–100 years, a more sustainable land use could allow to mitigate 5–14% of global carbon emissions by SOC sequestration. (Smith *et al.*, 2000, 2007; All, 2003; Faustian *et al.*, 2004). Clifton-Brown *et al.* (2004) estimated, with a simple model, that

about 12 Mt C y⁻¹ could be sequestered in EU-15 by growing *Miscanthus* on 10% of agricultural land, while Smith *et al.* (2008) indicated that SOC may account for up to 89% of the global potential mitigation for agriculture. Land conversion involving energy crops from surplus cropland resulted in 63% of the potential SOC sequestration in Europe (Smith *et al.*, 2000). It should be recognized; however, that SOC sequestration may increase only until an environmental equilibrium is reached or could even show a transient decrease followed by a complete recovery (West & Six, 2007). Converting grassland to *Miscanthus*, for example, was predicted to cause an initial SOC loss followed by a considerable carbon accumulation rate (Anderson-Teixeira *et al.*, 2009; Donnelly *et al.*, 2011). In a recent review on switchgrass, another dedicated perennial energy crop, Monti *et al.* (2012) reported that converting cropland to switchgrass generally increases soil C stocks at a

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rate of 1–1.2 Mg ha⁻¹ y⁻¹. Moreover, SOC levels will change with soil tillage, climate, soil type and agricultural management (All, 2003). In an extended review, Smith *et al.* (2008) reported that -0.25–1.30 Mg C ha⁻¹ y⁻¹ could be mitigated by adopting sustainable cropping practices, and 1.07–1.46 Mg C ha⁻¹ y⁻¹ by converting cropland to native vegetation.

To avoid conflict with food production, energy crops need to be planted on lower grade land unsuitable for arable crops such as wheat (Fargione *et al.*, 2008). Land abandonment may lead to negative effects on biodiversity, causing wild fires and decreased soil fertility (Peco *et al.*, 2012); keeping energy crops out of arable lands may reduce or avoid indirect land use change issues (Lemus & All, 2005; Field *et al.*, 2007; Fargione *et al.*, 2008; Frische *et al.*, 2010). It was estimated that in England and Wales, there are 870 000 hectares of marginal and 'idle' lands which could be used for bioenergy crop production, excluding areas of high biodiversity value (Haughton *et al.*, 2009; Turley *et al.*, 2010). However, if the development of energy crops is not properly regulated with regard to land allocation and use of the most suitable crop species, then the environmental and social benefits of biofuels may be substantially diminished. This could include possible conflicts between food and energy production and the consequent social and ethical issues that may arise (Field *et al.*, 2007; Rathmann *et al.*, 2010; Haberl *et al.*, 2011b).

Inappropriate choice of land types and crop types may even increase GHG emissions from soils such that the environmental benefits of growing bioenergy crops are negated (Fargione *et al.*, 2008; Hillier *et al.*, 2009; Frische *et al.*, 2010; Powlson *et al.*, 2011). Therefore, extending knowledge and understanding through quantification of soil carbon stock change under energy crops on different soil types such as poor quality arable or grasslands is crucial for the successful development of these crops and is of strategic value to policy makers.

Belowground biomass is the primary vehicle for soil carbon storage (Kuzyakov, 2002; Nguyen, 2003; Kell, 2011); therefore, perennial grasses are expected to increase soil carbon, mineralization processes being slower under minimal soil tillage and deeper root system (All & Kimble, 1997; Ma *et al.*, 2000; Monti & Zatta, 2009). Nevertheless, it is still questionable whether high root biomass corresponds to a proportionally high SOC accumulation. Some studies found that a large root biomass can trigger faster metabolic processes by soil microorganisms thus accelerating soil organic matter decomposition and C turnover, namely the 'priming effect' (Kuzyakov, 2002). Ultimately, a precise relationship between root biomass and SOC is not easy to establish as soil organic matter decomposition depends on several interacting factors including weather conditions,

soil characteristics, soil moisture content, oxygen concentration, microbial population and anthropologic factors such as soil tillage. For these reasons both losses and gains in SOC were observed in perennial energy grasses, such as switchgrass (Frank *et al.*, 2004; Monti *et al.*, 2012) and *Miscanthus* (Hansen *et al.*, 2004; Clifton-Brown *et al.*, 2007). Soil carbon sequestration under pasture management and in converting land use from pasture to forest was investigated in a number of studies (Gifford *et al.*, 1992; Conant *et al.*, 2001; Guo & Gifford, 2002; Paul *et al.*, 2002; Cowie *et al.*, 2006). SOC changes in converting arable land to *Miscanthus* energy crop tend to increase SOC to level similar to perennial grassland (Kahle *et al.*, 1999; Hansen *et al.*, 2004; Dondini *et al.*, 2009a,b; Zimmermann *et al.*, 2011; Felten & Emmerling, 2012), whilst changes from pasture to a *Miscanthus* energy crop has a small but ambiguous effect on SOC (Foereid *et al.*, 2004; Hansen *et al.*, 2004; Schneckenberger & Kuzyakov, 2007; Blagodatskaya *et al.*, 2011; Zimmermann *et al.*, 2011). Based upon documented measurements of SOC changes, Hastings *et al.* (2009) developed a simple model based upon the initial soil carbon before land conversion to *Miscanthus* and its annual harvested yield. Zenone *et al.* (2011) demonstrated using eddy covariance flux measurements that the process of converting grassland to soya crops, using herbicide to kill perennial grass and first tillage resulted in an extra respiration emission of between 1 and 4 Mg C ha⁻¹ in the year of conversion.

Miscanthus is one of the most promising candidate crops for energy-biomass across Europe (Lewandowski *et al.*, 2003; Tuck *et al.*, 2006; Stampfl *et al.*, 2007; Hastings *et al.*, 2009; Zegada-Lizarazu *et al.*, 2010). In the present study, we undertake to understand the fate of *Miscanthus* carbon input into former C3 grassland soil. We compared SOC stocks before and after a 6-year cultivation of *Miscanthus* genotypes planted on former grassland. To understand root biomass to SOC relationships belowground biomass was quantified orthogonally: vertically, at two different soil depths, and horizontally at three different positions from the centre of the plant. By analysing the ratio of stable carbon isotopes (O'Leary, 1988; Farquhar *et al.*, 1989) we estimated to what extent the priming effects counteracted the higher root biomass and finally we estimate the fate of soil carbon over the life cycle of a *Miscanthus* crop.

Materials and methods

Experimental field site and trial set up

The field experiment was conducted near Aberystwyth in Wales, UK (52°26'N, 4°01'W, 34 m elevation). The soil is classified as a dystric cambisol and a dystric gleysol depending on

spatial variation in drainage (FAO, 1988) with a stone fraction (particles >2 mm) of approx. 15% (0–30 cm soil layer). Soil texture was 18% clay, 24% silt and 58% sand. Wilt point and field water capacity were estimated to be 150 and 350 mm, respectively, using pedo-transfer functions (Campbell, 1985). This field has been part of the experimental station at Aberystwyth and has been used for trials for more than 30 years. It has been resown regularly (~5 years) with new grassland mixtures and used for silage and grazing tests. It has occasionally been used for arable plots of oats when flatter better land has been in short supply. Mature established perennial ryegrass was killed with Glyphosate (3 l ha⁻¹) in September 2004 and inversion tilled and resown in October 2004 with a ryegrass cover crop. This was subsequently sprayed with Atrazine (3 l ha⁻¹) on the 5th April 2005, 1 month before the timezero cores were taken (5 May 2005). The fragile biomass fragments were considered to be part of the soil and could not be separately quantified. The soil carbon stocks we determined at time zero are consistent with those expected of grasslands in this climate (Raich & Schlesinger, 1992; Parton *et al.*, 1995; Smith *et al.*, 2005). Four blocks of five 25 m² (6.67 m × 3.75 m) plots were marked out with 3 m paths between the blocks. Plots were separated by an equivalent of one planting row.

A tank mix of Atrazine (3 l ha⁻¹) was applied on 5 April 2005 to destroy the grass sward (*Lolium perenne*) in the plot areas. Before planting soil cores were extracted on the 9th May 2005 (more below). On 24th May 2005, four novel *Miscanthus* genotypes (Hy1-4, J. Clifton-Brown, unpublished results) which had been cloned by *in vitro* tillering were planted as bare root transplants of approximately 2 g fresh weight, in a similar manner to trees using a narrow spade. The control genotype, *Miscanthus* × *giganteus* Greef et Deu (Greef & Deuter, 1993; Hodgkinson & Renvoize, 2001) was planted similarly a few days later from fragments of clean overwintering rhizomes. Plants were planted directly (without soil cultivation) at a density of two plants m⁻². The carbon input from the propagules at planting was negligible (<20 g DM m⁻²). No fertilizer was applied over the 6 years, because soil analysis of the top 20 cm in November 2004 showed stocks were 6.7 Mg N (total) ha⁻¹, 34 kg P ha⁻¹ and 120 kg K ha⁻¹, sufficient to cover the requirement of the crop (Cadoux *et al.*, 2012).

Determining stock changes in soil organic carbon

Soil cores were taken to determine bulk density and soil organic carbon (SOC) on the 6th May 2005, before the *Miscanthus* were planted (T₀), and again after 6 years on the 5th May 2011 (T₆).

At T₀ two plots in each of the randomized blocks were randomly selected for coring. In each plot, five cores were taken in predetermined gridded positions with a 7.62 cm diameter corer with straight internal walls. To avoid compressing the sample (resulting in erroneous bulk densities) the corer was inserted and pulled back out every 5 cm down to a depth of 30 cm. Short 5 cm core samples were collated into one bag to make up 0–15 cm and 15–30 cm layers.

The *Miscanthus* hybrids tested here form tussocks making it more challenging at T₆ to take representative cores which can be scaled up to Mg SOC per hectare. To address this we

developed a more sophisticated sampling strategy that involved taking multiple cores at different positions with each plot. The coring positions were inter-row (C_i), edge of the plant (C_e) and centre of the plant (C_c) (Fig. 1). The tussock mass at C_c and C_e is made up of lignified rhizomes and stem bases which are too tough for hand coring. Based on field measurements, C_c, C_e and C_i accounted for 8.1%, 24.5% and 67.4% of the total field area respectively. The soil column cylinder auger (Eijkkelkamp, Giesbeek, The Netherlands) has been developed to take undisturbed soil samples. This corer has a cutting ring with a diameter of 8.5 cm and a depth of ~2 cm. After the ring, the internal diameter of the corer is slightly wider allowing the core to be supported, with minimal core sample compression. This allowed entire cores to be extracted from one insertion.

Ideally, soil bulk density would be constant for comparing C mass over time (Ellert *et al.*, 2001; Kimble *et al.*, 2001); however, it may change considerably with soil moisture, depth and physical properties (Harte, 1984; Ellert *et al.*, 2001). Moreover, due to soil tillage, soil mass may decrease from grassland to arable lands (Ellert & Bettany, 1995). By comparing soil height within the plant (C_c and C_e) and outside of the plant (C_i) it was estimated that rhizome growth displaced soil by 1–2 cm. To offset rhizome growth and resulting soil displacement, we sampled 1 and 2 cm deeper cores at C_e and C_c respectively. At C_i, the cores were taken without adding centimetres assuming that bulk density did not change appreciably as no tillage was made during the 6 year study (Powlson *et al.*, 2011). Cumulative mass coordinates is preferred to obtain a consistent comparison (Gifford & Roderick, 2003), although other authors have used spatial coordinates (Zan *et al.*, 2001).

In both T₀ and T₆, all samples were air dried until constant weight. In 2011, soil and belowground biomass were separated by hand. The air-dried soil was then passed through a 2-mm sieve to remove stones and any remaining and recoverable fine roots, the latter were added to belowground biomass. Belowground biomass was oven dried at 40 °C to constant weight.

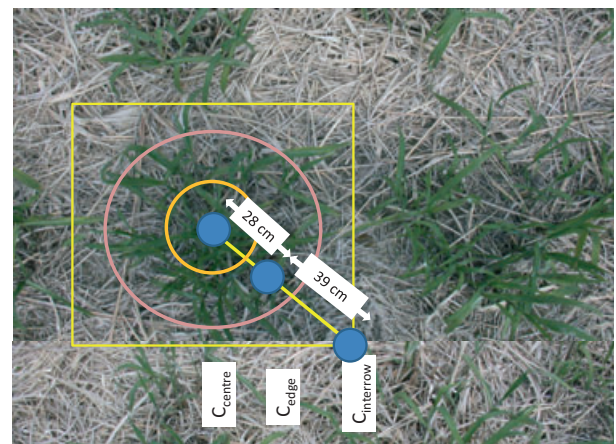


Fig. 1 Example of soil core samplings taken in each plot: interrow (C_i), edge of the plant (C_e) and centre of the plant (C_c). Photo 23 May 2012.

The *Miscanthus* contribution to soil carbon sequestration (F) was calculated using the following equation (Balesdent *et al.*, 1987):

$$F = \frac{(\delta_n - \delta_0)}{(\delta_r - \delta_0)}$$

where δ_0 and δ_n are soil organic C isotope abundance before planting of *Miscanthus* and after 6 years cultivation respectively; δ_r is the carbon isotope abundance of cryo-milled *Miscanthus* roots and rhizomes (three repetitions per hybrid). Soil carbon concentration (%) and stable carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$) was determined by an isotope ratio mass spectrometer (ANCA SL 20-20, Europa Scientific, Crewe, UK) in 250/300 mg soil samples, while the inorganic soil C content was determined by acidification of 3 g soil samples in 30 ml of HCl (1 mol l⁻¹) (Van Kessel *et al.*, 2000). Soil organic content (SOC) was calculated from the difference of total and inorganic soil carbon. The bulk density was calculated on the sieved dried soil (Ellert *et al.*, 2001). Carbon mass (M_c , Mg ha⁻¹) per unit volume was then calculated by multiplying soil bulk density (BD, Mg m⁻³), horizon thickness (T , m) and C concentration (C_{cont} , kg Mg⁻¹) as given by (Ellert *et al.*, 2001):

$$M_c = \text{BD} * C_{\text{cont}} * D * 10\,000 \text{ m}^2 \text{ ha}^{-1}$$

Modelling

The RothC model (Coleman & Jenkinson, 1999) was used to investigate dynamics of the soil carbon and predict the change in soil carbon changes over the life of a *M. × giganteus* plantation. Measurements of the *Miscanthus* yields were made annually in late February from 2006 to 2011. Aboveground biomass production in each replicate plot was measured by harvesting 15 m² in each plot using standard systems to determine moisture content on a subsample, which was then applied to calculate the dry matter (DM) at harvest per ha (Clifton-Brown *et al.*, 2001). The organic carbon input from the litter to the soil was calculated from the peak yield using the relationship proposed by Clifton-Brown *et al.* (2007), which is ripening loss for surface input of stem and leaves plus 10% peak yield for root turnover. The ratio of Carbon to DM was 0.59 (Table 1).

The previous land use was improved grassland for decades, RothC (Coleman & Jenkinson, 1999) was run to match the equilibrium using the mean meteorological conditions at the Aberystwyth site (Table 2) and assuming an annual input of 5.45 Mg C y⁻¹ and a recalcitrant plant matter/decomposable

Table 1 *Miscanthus × giganteus* dry matter harvest yield and estimated annual carbon input into the soil

| Year | Yield (Mg ha ⁻¹) | Carbon (Mg ha ⁻¹) |
|------|------------------------------|-------------------------------|
| 2005 | 0.3 | 0.07 |
| 2006 | 1.7 | 0.42 |
| 2007 | 10.9 | 2.77 |
| 2008 | 15.2 | 3.84 |
| 2009 | 13.9 | 3.53 |
| 2010 | 15.2 | 3.84 |
| 2011 | 17.2 | 4.36 |

Table 2 Mean climatic conditions (2005–2011) taken from Plas Gogerddan weather station near experimental field

| Month | Mean temp °C | Rainfall mm |
|-----------|--------------|-------------|
| January | 5.0 | 103.5 |
| February | 5.1 | 77.7 |
| March | 6.5 | 88.9 |
| April | 8.1 | 61.5 |
| May | 11.0 | 60.6 |
| June | 13.5 | 77.6 |
| July | 15.7 | 74.9 |
| August | 15.5 | 93.7 |
| September | 13.4 | 98.6 |
| October | 10.6 | 121.0 |
| November | 7.5 | 122.6 |
| December | 5.8 | 121.2 |

plant matter (RPM/DPM) ratio of 1.44 (Coleman & Jenkinson, 1999). For the year of conversion a C input of 1.5 Mg C y⁻¹ was included to account for the application of herbicide and the addition of the dead perennial grass roots and surface biomass which was included in the initial SOC sample. The model was then run for the period of the *Miscanthus* experiment using C input based on the annual yield and projected to the future with a constant yield of 16 Mg C y⁻¹ using the mean meteorological parameters.

The modelling was repeated for each hybrid using the measured difference between the peak summer yield and the winter harvest (ripening loss) to vary the input C for each hybrid. The ripening loss was compared to the measured SOC at 6 years.

The contribution of harvested biomass to CO₂ mitigation (C_s) compared with coal was calculated using the following equation:

$$C_s = \text{LHV}_m * \text{DM} * 0.033 \text{ kg C ha}^{-1}$$

where LHV_m is calorific value of *Miscanthus* (17.6 MJ kg⁻¹) (Collura *et al.*, 2006), DM is *Miscanthus* dry matter (kg ha⁻¹) and 0.033 kg C MJ is the energy intensity of coal (Hastings *et al.*, 2009).

Data analysis

All data were subjected to the analysis of variance (ANOVA). When ANOVA revealed significant differences ($P \leq 0.05$), the Tukey's LSD test was used to separate means (CoStat v6.204, Monterey, USA). In text, means are presented with \pm standard deviation unless otherwise specified.

Results

Belowground biomass

The term belowground biomass as used here refers to all roots and rhizomes. As expected, in the 0–15 cm layer, due to a higher rhizome component at C_c and to a lesser extent at C_e , the belowground biomass per volume of soil, or belowground biomass density, were

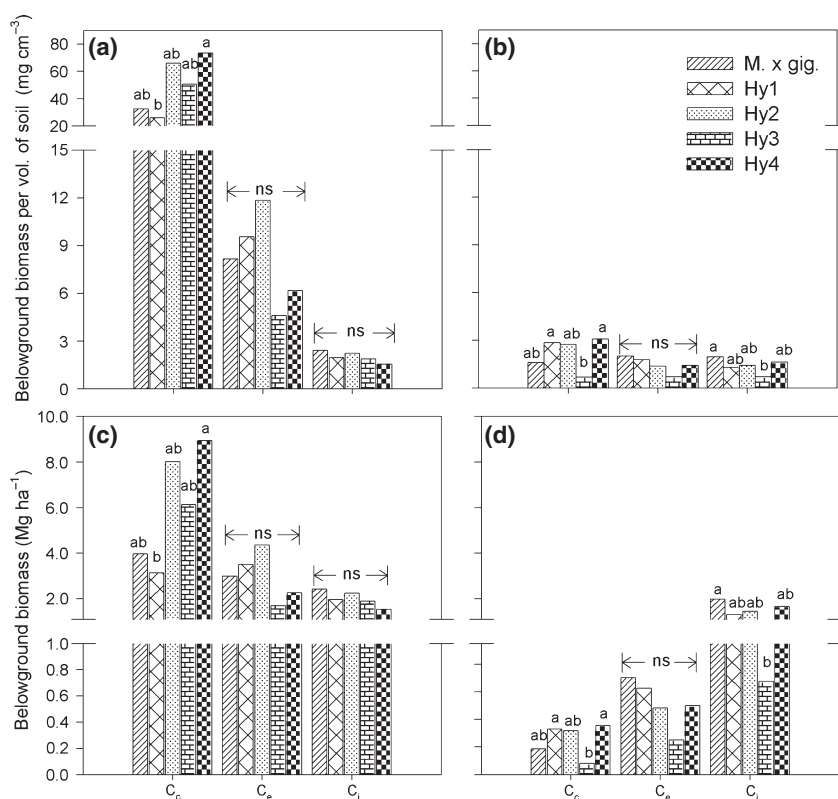


Fig. 2 Belowground biomass (roots plus rhizome) of *Miscanthus* hybrids at C_c, C_e and C_i (centre, edge and interrow, respectively, see Fig. 1) at two soil depths: 0–15 (a) and 15–30 (b) cm. The belowground biomass per hectare contributed by C_c, C_e and C_i (Fig. c, d) were calculated using the corresponding areas represented by each core position in one hectare (8.1%, 24.5% and 67.2%, in that order). Different lower case letters show statistically different means (Tukey's LSD test, $P \leq 0.05$) within a core position. ns = not significant.

clearly different at C_c, C_e and C_i (Fig. 2a). Hybrids did not generally differ in belowground biomass density; the only exception was at C_c between Hy1 and Hy4 (Fig. 2a). *Miscanthus* genotype showed some significant differences even in the deeper layer, however, these differences were not as large as in the upper one (Fig. 2b).

Therefore, given the belowground biomass densities, we calculated the belowground biomass (Fig. 2c and d). In the upper layer, belowground biomass still showed the highest values in C_c, while unlike density, C_e and C_i showed a similar biomass values (Fig. 2c). By contrast, at the deeper layer, the belowground biomass exhibited a reverse trend to biomass density, thus resulting in a quite similar biomass among C_c, C_e and C_i over the 0–30 cm soil layer (Fig. 2d).

Estimating the soil organic carbon content

The development of roots and rhizomes, especially in young plants, can be expected to significantly reduce soil bulk density (BD); therefore, to collect an equivalent soil mass after 6 years we sampled 1- and 2-cm longer soil cores in correspondence of C_e and C_c respectively. The results showed that BD significantly decreased after

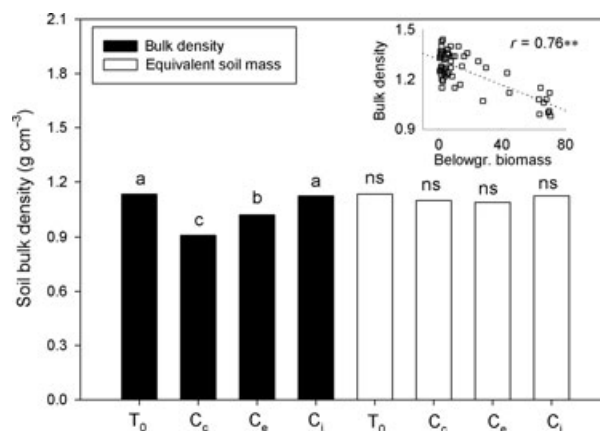


Fig. 3 Soil bulk density of the cropland (T₀) and after 6 years of *Miscanthus* (graph A) at C_c, C_e and C_i (centre and edge of the plant and interrow respectively) upper layer. The equivalent soil mass (graph B) refers to the real amount of sampled soil as 1 and 2-cm longer cores were taken at C_e and C_c, respectively, to offset the decrease of bulk density due to *Miscanthus* root and rhizome development. The inset graph shows the effect of the belowground biomass development on bulk density. Different letters indicate statistically different means within filled and unfilled bars (Tukey's LSD test, $P \leq 0.05$).

6 years (Fig. 3 inset). However, it was only significant at locations C_c and C_e (Fig. 3). Based on the assumption that average biomass density would not appreciably change between two soil profiles of 17–18 cm (i.e. 15–32 and 15–33 cm soil layers) we recalculated the equivalent soil mass considering a soil core of 18 cm. As this equivalent soil mass was not different from T_0 we added the amount of belowground biomass equivalent to that contained in 1 cm of soil according to the real average belowground biomass (that measured between 15 and 32 cm of depth) (Fig. 3).

Although soil C depletion caused by land use change from grassland to *Miscanthus* was evident in all hybrids with a range between -5 (Hy1) and -10 (Hy2) Mg C ha^{-1} (Fig. 4), that decrease was not statistically significant when compared to T_0 . Therefore, based on field measurements in which C_c , C_e and C_i accounted for 8.1%, 24.5% and 67.4% of total area, we could not detect an overall reduction in SOC after 6 years (Table 3). Significant SOC decreases were only found at C_e and C_c for Hy2 (Fig. 4).

By the use of carbon isotope technique we could determine the *Miscanthus*-derived C, i.e. the contribution of *Miscanthus* to total SOC after 6 years. All the hybrids contributed a similar amount of C, which decreased from C_c to C_i , averaging 14% and 9.9% in the upper and deeper layers respectively (Fig. 5). *Miscanthus*-derived C positively correlated with belowground biomass (Fig. 6); however, the statistically insignificant change of total SOC (Fig. 4) might lead one to expect a triggering effect on soil respiration rates and C turnover by higher root and rhizome deposition or by exudates and organic substances produced by living roots, namely the rhizo-

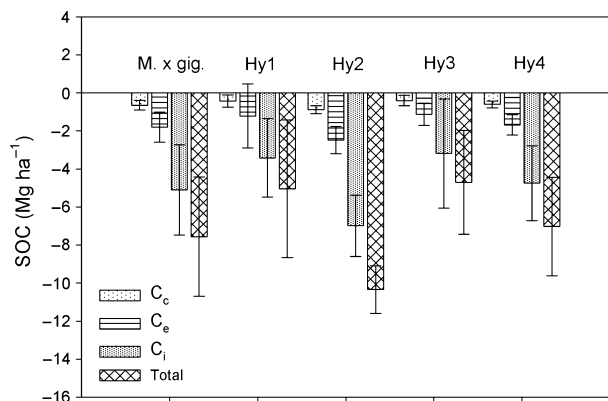


Fig. 4 Differences between soil organic carbon (SOC, Mg ha^{-1} , 0–30 cm) after 6 years of *Miscanthus* hybrids (*Miscanthus* \times *giganteus* and Hy1 to 4) and SOC of the grassland just before *Miscanthus* plantation in the same profile (T_0). C_c , C_e and C_i indicate the amount of SOC at plant centre, plant edge and interrow respectively. At T_0 , SOC was $78.8 \text{ Mg C ha}^{-1}$. Bars indicate standard error ($n = 4$).

Table 3 Analysis of variance: effects of depth and hybrid, between T_0 and T_6 , on measured soil parameters (* and **, statistically significant differences for $P \leq 0.05$ and $P \leq 0.01$ respectively)

| Soil core position | Soil parameter | Depth | Hybrid | CV |
|-------------------------------|------------------|-------|--------|------|
| Centre of the plant (C_c) | BD | * | ns | 11.0 |
| | C_{mis} | ** | ** | 2.8 |
| | SOC | ** | ns | 12.4 |
| Edge of the plant (C_e) | BD | ns | ns | 9.4 |
| | C_{mis} | * | ** | 1.9 |
| | SOC | ** | ns | 11.8 |
| Interrow (C_i) | BD | ns | ns | 8.3 |
| | C_{mis} | ns | ** | 1.3 |
| | SOC | ** | ns | 12.9 |

BD, bulk density; SOC, soil carbon content; C_{mis} , *Miscanthus* derived C; CV (%), coefficient of variation. Depth \times hybrid interaction was never significant.

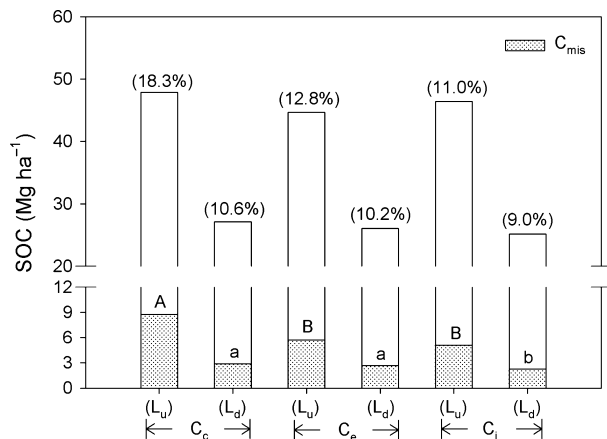


Fig. 5 *Miscanthus* derived C (C_{mis}) on total soil organic carbon (SOC) in the upper (0–15 cm, L_u) and deeper layer (15–30 cm, L_d). C_c , C_e and C_i indicate centre and edge of the plant and interrow respectively. Different letters indicate statistically different C_{mis} in the two soil layers (Tukey's LSD test, $P \leq 0.05$), uppercase letter for L_u , and lower case letter for L_d . Numbers between brackets indicate the percentage of C_{mis} on total SOC.

sphere priming effects (Fig. 6). In Figure 7, the absolute amount of SOC in the C_c upper layer of soil after 6 years correlates negatively with the below ground biomass providing some evidence for this priming effect as the C3 C is apparently replaced by the C4-C faster, but this is not reflected in the overall SOC values.

Modelling SOC

The RothC modelling results show the initial equilibrium for soil carbon on the perennial C3 grassland was approximately 77 Mg C ha^{-1} . This is increased before

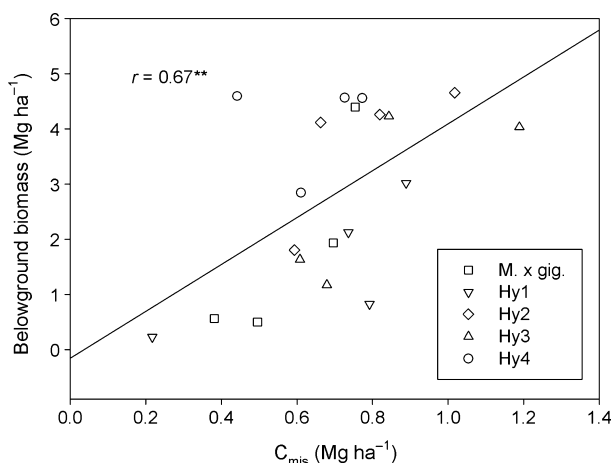


Fig. 6 Correlation between belowground biomass and *Miscanthus* contribution to total SOC (C_{mis}) in the upper layer (0–15 cm) after 6 years of five *Miscanthus* genotypes (*Miscanthus* × *giganteus*, Hy2 to 4) grown in a former grassland in Aberystwyth, Wales, UK.

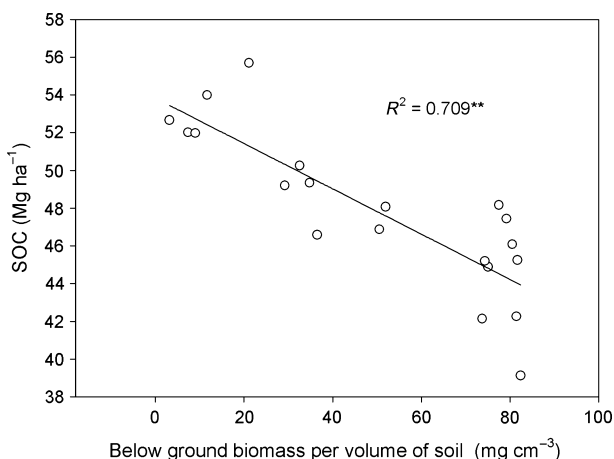


Fig. 7 Correlation between belowground biomass intensity and soil organic carbon (SOC) in the upper layer (0–15 cm) after 6 years in the centre of the plant (C_c).

planting the *Miscanthus* to 78.8 Mg C ha⁻¹ due to the input of herbicide killed C3 grass biomass. The original C3 origin C decays by 14.7 Mg ha⁻¹ between planting to sampling in 2011, whilst the C4 input adds 7.5 Mg C ha⁻¹. The model results agree with the observations within the SE. Projections to 2025 show that the SOC remains constant with the C4 origin carbon replacing the C3 carbon, giving a similar overall level of SOC (Fig. 8). The final SOC for each of the hybrids correlates positively with mean difference between peak summer and harvest yield (ripening loss) with a $R^2 = 0.663$ ($n = 5$), indicating that this is the dominant explanatory variable but that there is probably a small non-quantified variability in C input lability between the hybrids (Fig. 8 insert).

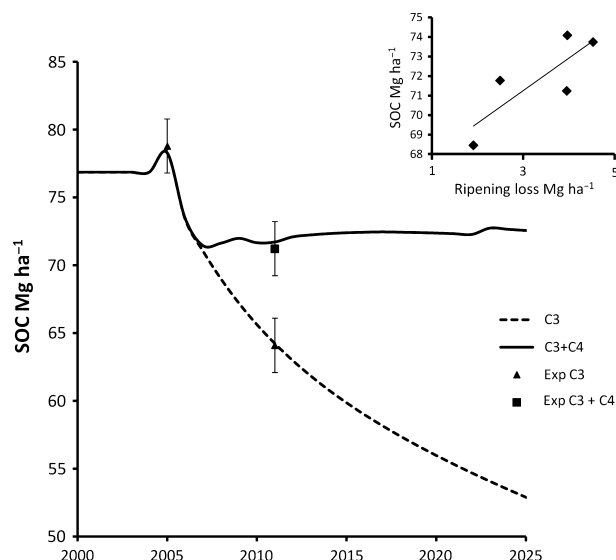


Fig. 8 Results of RothC simulations of the decomposition of the original soil organic carbon (SOC)(C3) and total soil carbon including the *Miscanthus* C input (C3 + 4) using mean meteorological conditions for the site for both historical input and projected for a plant life of 20 years. This is compared temporally to the SOC measured before *Miscanthus* planting but after the herbicide killed the original C3 perennial grass and the measurements in 2011 of the total soil carbon (Exp C3 + 4) and minus the contribution of the *Miscanthus* input (Exp C3). Main plot shows the simulation for *Miscanthus* × *giganteus* and the inset shows the relationship between ripening loss and final SOC for all hybrids.

Discussion

SOC quantification by coring

For practical reasons soil sampling in row crops is often simplified by only sampling between the rows (Zan *et al.*, 2001; Frank *et al.*, 2004; Monti & Zatta, 2009). To obtain the ‘overarching’ SOC estimates reported in this paper we developed a novel sampling strategy to overcome the technical challenges of representative sampling in a tussock forming plant such as *Miscanthus*.

Our three core method (Fig. 1) with proportional representation of plant centre, plant edge and inter-row allows defensible up-scaling to units such as Mg of SOC and below ground biomass per hectare. We developed this method to avoid significant damage to the plots caused by digging out entire quadrates (Clifton-Brown *et al.*, 2007). We intend to make further similar samplings at T₁₂ and possibly T₁₈ so that we can understand carbon dynamics over the likely useful life-span of the crop (currently estimated to be up to 20 years).

Evidence for microbial 'priming' effects

A positive correlation between belowground biomass and SOC might be expected (Ma *et al.*, 2000; Lemus & Lal, 2005; Field *et al.*, 2007; Monti & Zatta, 2009). The *Miscanthus* hybrids in our experiment accumulated significantly different quantities of belowground biomass, but this was not reflected in the total SOC after 6 years. Curiously the genotype with the highest belowground biomass (Hy2), led to the highest SOC reduction from the values measured at the start of the trial (Fig. 4). The absence of a significant correlation between increase in SOC and belowground biomass might be explained by a triggering effect of belowground biomass on soil metabolism, namely the 'rhizosphere priming effect'. This attempts to explain the faster decomposition of SOC by micro-organisms in response to a higher fresh organic matter supply (Kuzaykov, 2002, 2006). This hypothesis seems to be corroborated by a significant correlation between belowground biomass and *Miscanthus*-derived C in C_c upper layer (Fig. 5), suggesting that a priming effect occurred in the direct vicinity of living roots (Kuzaykov, 2002). SOC depletion observed in Fig. 4 could be explained by increased priming effect due to high organic matter (Mary *et al.*, 1993; Asmar *et al.*, 1994) causing increased mineralization which has been reported to reach up to 400% (Kuzaykov, 2002). Therefore, soil respiration may correlate with biomass deposition rates, and where belowground biomass accumulated in greater amounts it was also degraded more rapidly. It is unclear why the extent of this priming effect varied with *Miscanthus* genotype; for example Hy4 produced higher root biomass than Hy2, 65.5 and 58.6 mg m⁻³, respectively, but the latter showed a higher contribution (+5%) to SOC. A possible explanation could be that priming effects were driven by variable amounts of more labile organic substances (e.g. polysaccharides, carbohydrates and celluloses) or recalcitrant (e.g. lignin, waxes and suberins) carbon pools deriving from belowground biomass (Nguyen, 2003; Jones & Donnelly, 2004; Fioretto *et al.*, 2005; Kuzaykov & Larionova, 2005; Jastrow *et al.*, 2007; Lal, 2008). In conclusion, possibly due to priming effects, belowground biomass seems, by itself, not sufficient for predicting SOC dynamics. Further studies are required to understand better the proportion of autotrophic and heterotrophic soil respiration underlying our observations in SOC dynamics.

Soil carbon stocks and the saturation point

Another possible explanation of the unexpected association between SOC variation irrespective of genotype

and belowground biomass could be the saturation of SOC level, implying that C stock was saturated with respect to C inputs (Freibauer *et al.*, 2004; Stewart *et al.*, 2007; Powlson *et al.*, 2011). The potential for soil to sequester C is linked with regional climate, soil properties and land management (West & Six, 2007) and it is known that grasslands tend to have high SOC content (Guo & Gifford, 2002). This hypothesis seems, however, in contrast with the considerable variation of SOC found in C_i, C_e and C_c, that showed SOC values from 2.2% to 3.3% in the upper layers, and from 0.8% to 2.1% in the deeper layers thus suggesting that C stock in the soil was not saturated. We might expect, in subsequent samplings after longer time periods (e.g. 12, 18 years) that SOC levels would correlate with differences in carbon partitioning of the genotypes. For example, in Denmark soil organic matter remained relatively constant for the first 11 years following establishment with *M. × giganteus* on a grassland site (Foereid *et al.*, 2004). However, a period of 20 years is needed to provide the real carbon sequestration by the soil (Houghton *et al.*, 1997).

Model history match and predictions

The RothC model indicated that the *Miscanthus* plot behaviour is similar to perennial C3 grassland as the lability of the C4 C input was kept the same as the default used for temperate C3 grassland (RPM/DPM ratio of 1.44). Ultimately the SOC equilibrium will be a function of quality of C input each year and its decomposition rate. The predicted equilibrium for the *M. × giganteus* plot SOC seems to be similar to the original C3 perennial grassland. Modelling runs for each hybrid shows similar results with a strong correlation between the final SOC at 6 years and the measured ripening loss confirming this hypothesis. There appears to be a small difference in lability between the hybrids as the RPM/DPM ratio has to be modified slightly to get a perfect match. It should be noted that the management has an impact, because the killing of the C3 grass by a herbicide results in an input of 1.5 Mg C ha⁻¹ momentarily increasing the SOC at the time of sampling. This is not compensated by the small C input from the *Miscanthus* plants during the establishment years resulting in the observed reduction in SOC. The subsequent mature plant input rate of C4-C is not quite enough to compensate for the C loss in the land use change. However actual predicted change is small and the run made with the higher yielding hybrid results in the same SOC after 20 years. From this we conclude that there is a very small to neutral C emission from the land use change from grassland to *Miscanthus* and it is dependent on the hybrid used.

Impacts of land use change from grassland to *Miscanthus* on SOC

There is evidence that conversion of some land uses to energy crops, particularly the annuals, may cause significant SOC losses (Lal, 2004; Fargione *et al.*, 2008; Searchinger *et al.*, 2008). The conversion of natural to agricultural ecosystems, for example, led to a SOC depletion of 60% in temperate regions, and up to 75% in tropical regions (Lal, 2004). A decrease of SOC was also found when energy crops were planted on forest lands (Murty *et al.*, 2002), peatlands (Page *et al.*, 2002; Inubushi *et al.*, 2003), savanna (Fargione *et al.*, 2008) or former grasslands (Follett, 2001; Tilman *et al.*, 2006). However, St. Clair *et al.* (2008) included land use change and its associated soil carbon change in a life cycle analysis of energy crops and suggested a neutral effect of planting *Miscanthus* on grassland. In contrast, perennial grasses planted on arable lands considerably increased soil carbon reserves (Kort *et al.*, 1998; Field *et al.*, 2007; Lee *et al.*, 2007; Fargione *et al.*, 2008; Anderson-Teixeira *et al.*, 2009; Hillier *et al.*, 2009; Monti & Zatta, 2009). SOC increased up to 18% under a 3 year-old switchgrass stand (Zan *et al.*, 2001), and up to 29% under a 16 year-old *Miscanthus* stand (Hansen *et al.*, 2004) both planted on croplands. In the future, with increasing population and food production requirements the main land resource for energy crops will be lower grade agricultural land often not used for arable crops (Haberl *et al.*, 2011a).

In Wales and England it is estimated that 870 000 hectares of marginal and 'idle' lands, excluding areas of high biodiversity value, are potentially available for bioenergy crop production (Turley *et al.*, 2010). Data from the present 6 year study, will reassure policy makers that planting on these semi-permanent grasslands with a range of *Miscanthus* genotypes did not deplete SOC significantly over the 6 years. It is highly unlikely with increasing stand age that SOC levels will deplete relative to T_0 , and following the trends from arable land, it is likely there is some scope for SOC increases up to the soil type-environmental equilibrium (Jones & Donnelly, 2004; Powlson *et al.*, 2011). There is undoubtedly some value of this small but significant carbon sequestration sink, which we hope to quantify in years to come.

The immediate carbon benefits of *Miscanthus* cultivation are the substitution of fossil carbon sources when the crop is used to produce energy. *Miscanthus* biomass is a solid fuel, and therefore it is reasonable to use it to substitute coal. Combining accurate yield records from annual harvests made in February (unpublished) and the calorific value of these *Miscanthus* genotypes [Hodgson, unpublished, but it is close to published values of 17.6 MJ kg⁻¹ (Collura *et al.*, 2006)] we can calculate the

carbon substitution benefit. These figures show for the five genotypes over the 6 years that the 'coal' carbon substitution ranged from 70 (Hy3) to 103 (Hy1) Mg CO₂ ha⁻¹. Adding in the belowground C content (SOC and roots and rhizomes), the total C saved ranged from 73 (Hy3) to 109 (Hy2) Mg CO₂ ha⁻¹. We conclude the carbon benefit of growing *Miscanthus* as an energy crop on improved grasslands in the UK was largely from fossil fuel substitution. This study was over 6 years, growing *Miscanthus* for longer periods may slightly increase the role of soil carbon sequestration, but is unlikely to be significant in the overall carbon mitigation benefit when planted on improved grassland in the UK.

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