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Sequestration of C in soils under *Miscanthus* can be marginal and is affected by genotype-specific root distribution

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ABSTRACT

Miscanthus is a low input energy crop suitable for low fertility marginal arable land and thought to provide carbon sequestration in soil. We analysed a long-term field experiment (14-year) to determine whether differences in genotype, growth habit, and root distribution affected soil carbon spatially under different *Miscanthus* genotypes. Soil cores were taken centrally and radially to a depth of 1 m, and divided into six vertical segments. Total root length (TRL), root dry matter (RDM) and $\delta^{13}C$ signature of soil organic carbon (SOC) were measured directly, and root length density (RLD), fractions of *Miscanthus*-derived soil organic C (SOC_M), and residual soil carbon (SOC_{orig}) were calculated. Genotype was found to exhibit a statistically significant influence on spatial allocation of SOC. Grouping varieties into 'tuft-forming' (T) and 'non-tuft-forming' (NT) phenotypes revealed that respective groups accumulated similar amounts of RDM over 14 years (11.4 ± 3.3 vs. 11.9 ± 4.8 Mg ha^{-1}, respectively). However, phenotype T allocated more carbon to roots in the subsoil than NT (33% vs. 25%). *Miscanthus* genotypes sequestered between 4.2 and 7.1 g C_4-SOC kg^{-1} soil over the same period, which was more than the average loss of C_3-derived SOC (3.25 g kg^{-1}). Carbon stocks in the 'A horizon' under *Miscanthus* increased by about 5 Mg ha^{-1} above the baseline, while the net increase in the subsoil was marginal. Amounts of *Miscanthus* root C in the subsoil were small (1.2–1.8 Mg C ha^{-1}) but could be important for sustainable sequestration as root density (RLD) explained a high percentage of SOC_M ($R^2 = 0.66$).

1. Introduction

Miscanthus is a favored perennial feedstock for bioenergy in subtropical and temperate regions due to its high potential productivity (Heaton et al., 2010; Lewandowski et al., 2003) and benefits with regard to the carbon and greenhouse gas balance (Dondini et al., 2009; Hillier et al., 2009). Domestication of these perennials is in its infancy and genotypes may be found or bred that suit a wider range of ecological conditions and maximize efficiency of carbon sequestration (Clifton-Brown et al., 2008; Karp and Shield, 2008; Yan et al., 2012). The increasing interest in *Miscanthus* (e.g. Stewart et al., 2009; Yan et al., 2012; Sang and Zhu, 2011) should be accompanied by the exploration of the carbon budgets of other genotypes in addition to commercially grown *Miscanthus* × *giganteus*. This would clarify whether contrasting *Miscanthus* phenotypes (growth habit, rooting pattern) act as an effective sink (Qin et al., 2011) or even a source (Yazaki et al., 2004) of atmospheric carbon. Key considerations in determining the soil organic carbon (SOC) balance require measurement of the C fraction deposited into the subsoil, which is less likely to be remobilized than C deposited in the surface horizon (Kell, 2011; Lockwell et al., 2012). Measurements of ^{13}C abundance can also be used to indicate the stability of these inputs in the surface and subsoil under commercially grown *Miscanthus* × *giganteus* (e.g. Zimmermann et al., 2013). Existing studies of the genotype effect focus on carbon near the surface (Zatta et al., 2014) which ignores the potentially beneficial effect of deep roots as a mechanism to sequester carbon (Kell, 2011).

It is of further interest how contrasting growth forms, e.g. phenotypes (tuft or non-tuft) and carbon allocation patterns, e.g. different above- and belowground biomass allocation (AGB and BGB, respectively), and root densities, affect SOC. An integrative comparison of genotypes can inform about the relationships between productivity, carbon partitioning and

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Abbreviations: AGB, above ground (dry) biomass (Mg ha^{-1}); BGB, below ground (dry) biomass (Mg ha^{-1}); G, gap; P, plant; RLD, root length density (cm cm^{-3}); RDM, root dry matter (g m^{-2}); RD, root diameter (mm); SOC, soil organic carbon (%); TRL, total root length (km m^{-2}); T, tuft forming *Miscanthus* genotypes; NT, non-tuft forming *Miscanthus* genotypes.

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carbon sequestration characteristics, including vertical and lateral root distribution in response to rhizome form and size. This may have practical implications, such as elucidating the potential for increasing sequestration by breeding or selecting varieties with deep roots (Kell, 2011).

The relative contributions of AGB and BGB are easily confounded (Cotrufo et al., 2010) with annual litter inputs from *M.* × *giganteus* being between 1.5 and 7 Mg ha^{-1} yr^{-1} (Beuch et al., 2000; Kahle et al., 2002; Amougou et al., 2011). The contribution of roots to SOC is thought to be significantly greater than that of litter in grassland (Gill et al., 2002) and woody ecosystems (Rasse et al., 2005). Clifton-Brown et al. (2007) estimated that the C sequestered from *Miscanthus* into SOC after 15 years was equal to 10% of the BGB assuming a total input of 20 Mg dry weight ha^{-1}, which contributed 14% to the total SOC in the first 10 cm layer. In deep soils the *Miscanthus* root-fraction was shown to accumulate initially at the net rate of >2 Mg ha^{-1} yr^{-1}, which then decreased to about 1 Mg ha^{-1} yr^{-1} as a result of >3 Mg ha^{-1} growth and >2 Mg ha^{-1} decomposition (Neukirchen et al., 1999). In the present work, we aim to characterise the distribution of *Miscanthus*-derived SOC (SOC$_M$) throughout the soil profile with particular attention to contrasts between individual genotypes from the main phenotypic growth forms (tuft vs. non-tuft; see definition below), and relate these differences to measurements of root distribution.

Starting from a solely C$_3$-cropped site we use the *Miscanthus* induced change in δ^{13}C signature to distinguish between the original C$_3$-based organic carbon (SOC$_{orig}$) and SOC$_M$ under contrasting genotypes. From these quantities we then estimate C sequestration throughout the soil profile (depth 1 m) and relate this to the rooting and growth patterns of these genotypes on a marginal arable soil under low nitrogen (N) input and climatic conditions typical of the site at Rothamsted, UK.

2. Materials and methods

2.1. Field experiment and genotypes

The field experiment used in this study was established in 1997 as part of the European *Miscanthus* Improvement (EMI) program conducted at five locations in Europe (Clifton-Brown et al., 2001). The EMI field trial in England was established on a long-term arable field at Rothamsted Farm (51.81 N–0.358 E) on a silty clay loam with sandy inclusions (Batcombe–Carstens series; chromic luvisol or aquic paleudalf). C$_3$ annual cereals and break-crops were grown exclusively on both the *Miscanthus* (Long Hoos III) and reference arable sites (Long Hoos IV) and conventionally tilled for 50 years or more (Johnston et al., 1981). The reference had remained under continuous arable management for all years since the *Miscanthus* was planted. The N input to a mixed arable crop rotation averaged 141 (range 80–190) kg N ha^{-1} yr^{-1}. The *Miscanthus* genotypes were planted as micro-propagated plantlets in 5 m × 5 m plots at a density of two plants per square meter in late May 1997. The trial had a fully randomised block design with three replicates. Plants had been drip irrigated (+273 mm above the natural rainfall) during the first year. Details of fertiliser applications and management can be found in Riche et al. (2008). Over 14 years approximately 50 kg N ha^{-1} yr^{-1} was applied to support increasing annual yields between 4.8 and 15.9 Mg ha^{-1} yr^{-1} which then declined and accumulated totals of 100–123 Mg ha^{-1} (Table 1).

Out of the 15 genotypes included in the EMI program we selected five genotypes that represent four genetic groups (Table 1): (1) *M.* × *giganteus* (Gig-1) is a vigorous natural hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*, widely grown commercially in UK and Europe, (2) *M. sacchariflorus* (Sac-5) is also grown in central Europe, originally obtained from Japan in 1992, (3) and (4) are two genotypes (SinH-6 and SinH-9) from the *M. sinensis* hybrid collection, which are characterised by a higher leaf fraction (36–62%) and yield reduction under drought, (5) Sin-11, a *M. sinensis* from Japan, which showed the least yield variation among the chosen genotypes.

These genotypes can also be grouped according to their aboveground growth habit or rhizomes (Lewandowski et al., 2003). *M. sacchariflorus* has broad, thick-stemmed rhizomes which creep laterally from where shoots develop out of internodal buds (non-tuft, NT phenotype) while rhizomes of *M. sinensis* genotypes are much smaller, do not exhibit the lateral creeping habit and aboveground shoots form dense centralised tufts made out of thinner stems (tuft, T phenotype). The annual dry matter allocation to rhizomes was estimated from earlier whole plant analysis and excavations. Based on the much larger fraction of rhizome accumulated under NT than T genotypes (>30 vs. <10 Mg ha^{-1}, respectively; Table 1) one could consider this an important phenotypic trait. The rhizome fraction ranged from 23% for Sac-5 to between 6 and 11% of total accumulated yield for the *M. sinensis* genotypes. The hybrid, *M.* × *giganteus*, allocates *circa* 15% of the C to intermediate rhizomes, which creep less than *M. sacchariflorus* (Table 1). For investigating the effect of this phenotype contrast we grouped these into tuft forming (T; Sin-H6, Sin-H9, Sin-11) and non-tuft forming groups (NT; Gig-1 and Sac-5).

2.2. Soil sampling and preparation

A corer with an inner sleeve that could be dismantled longitudinally (diameter 70 mm; length 1 m) was driven into the soil using a hydraulic jackhammer and extracted using a tripod ratchet. Two cores were taken from each plot to a depth of 100 cm, one central to the original planting site (P) and one between plants, in the gap (G) situated midway between plants (32.5 cm from position P). Cores were wrapped in polythene and stored at −18 °C pending root and soil analyses. A further three random cores were taken from the adjoining arable reference site, approximately 10 m from edge of the EMI trial as reference points for δ^{13}C and total C (hereon termed 'Reference Arable'). An equivalent soil mass (ESM) of the A horizon (0–30 cm) of the *Miscanthus* plots was found in the 0–26 cm layer of the Reference Arable soil (due to *Miscanthus*

Table 1
Growth characteristics of Tuft- and Non-Tuft (T, NT) forming *Miscanthus* genotypes used in the root and carbon analysis; harvested yields (min, max) and cumulative production (dry matter) over 14 years after planting; litter residues and rhizome dry matter accumulated at time of hand harvest (April 2011). Fractional area of tuft derived from circumference (m^2 m^{-2}).

Genotype		Phenotype	Tuft size (–)	Yield (Mg ha^{-1} yr^{-1})		Culmuative biomass (Mg ha^{-1})		
				Min	Max	Harvest	Litter	Rhizome
M. giganteus	Gig-1	NT		5.6	15.9	120	9.3	33
M. sacchariflorus	Sac-5	NT		7.5	15.4	123	8.5	29
M. sinensis hybr	Sin-H6	T	0.56	6.5	13.9	114	3.3	9
M. sinensis hybr	Sin-H9	T	0.44	4.8	15.3	114	8.3	7
M. sinensis	Sin-11	T	0.46	6.1	10.8	101	8.9	11