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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

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 $\delta^{13}C$ 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 $\delta^{13}C$ 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

reducing the bulk density, especially in the top 10 cm). In addition to the reference samples, archived samples from the site were retrieved for the period prior to planting the *Miscanthus* (1988) and analysed to obtain the baseline SOC content. This archived material always used to be sampled to a depth of 0–23 cm because this represented former ploughing depth.

The cores were cut into three sections for each horizon composed of topsoil (A; depths 0–10, 10–20, 20–30 cm) and subsoil (B; 30–50, 50–70, 70–100 cm), respectively. Due to high soil moisture at sampling, the cores were variably compressed, two thirds between 0 and 5%, and only three cores were exceeding 10% compression. A proportional adjustment was made for all sections of the compressed core before division. These sections were then divided into approximately equal half-cores and each half was weighed. One half-sample was kept for root washing while the other half was air-dried for the determination of soil moisture and chemical analysis (see below). Stones were removed from both sub-samples. Dry bulk densities were determined from each segment using the volume and stone-free dry matter content to estimate the carbon content at each depth. The air-dried soils were gently separated from visible organic matter, litter, roots, rhizomes and large stones (flint, chalk). The soil was then sieved (<2 mm) and crushed using a disk mill (TEMA Machinery Ltd.).

2.3. Root extraction and characterisation

The root core half section was placed in a bowl of warm water to gently tease the soil apart from *Miscanthus* structures, and then to carefully separate plant roots from rhizomes and litter debris. Large roots were collected on a fine sieve (0.25 mm) to enable soil dispersion and small soil particles to be removed, and roots were then placed straight into a water-filled glass jar. Rhizomes and aboveground plant materials were removed from the top section of each core. Once the visible roots had been removed, the content of the bowl containing the fine roots was poured onto the sieve and rinsed thoroughly with water to remove any soil. Roots were subjected to a second rinse if the sample was not clean (i.e. containing chaff or clay) and then combined and stored in aqueous 10% ethanol in plastic bottles and kept in a dark cool room (4° C) before scanning.

2.4. Root scans and image analysis

Root samples were spread on an A4 plexiglass water tray of the WinRhizo flatbed scanner (Epsom STD 4800). Root length and diameter were quantified using the WinRhizo Pro (2008) software package, applying a standard set of acquisition parameters for black and white (grey shades) for the root length and diameter classes. Scans were saved as tiff files pending further image analysis. After scanning was complete, the roots were dried at 40 °C to determine the root dry matter (RDM; g). This was then converted to RDM per depth increment (RDM(z); Mg ha^{-1}) using the respective stone-free dry bulk density of the cores (ρ_{SF}). Root length density (RLD) was calculated from the measured total root length (TRL) and the volume of the stone-free soil in each segment.

2.5. Carbonate and its removal

Carbonates in the soil from underlying chalk or added lime interfere with the determination of $\delta^{13}C$ of soil organic matter (SOM) because they exhibit a $\delta^{13}C$ signature close to that of PDB (around 0‰). Therefore, carbonates were removed before isotope analysis by acid treatment. To avoid loss of soluble organic C by acid washing, Harris et al. (2001) proposed to expose moistened soil to concentrated HCL vapor (12 M) for 6–8 h before isotope ratio mass spectrometry (IRMS). However, this popular technique was found to deposit strongly acidic residues that remained even after repeated application of vacuum (not shown). The residual HCL appeared to interfere with the analyses and damage the mass spectrometer. We therefore applied the following method, similar to that developed for the removal of carbonates from coastal sediments (Komada et al., 2008), which combines the advantages of utilising an invasive aqueous phase without losing soluble organic C or accumulating problematic quantities of HCL.

Subsamples of 20.00 ± 0.20 mg milled soil were weighed into A-foil capsules (9 mm) and placed in a random arrangement on micro-titre plates (F96 Maxisorp, Nunc, Denmark) allowing adequate space between each sample. To each capsule, sufficient aqueous solution of trace analysis grade HCl (1 M) was added to bring the soils approximately to field capacity (in this case 35 μL). The plate was subsequently placed in an empty, carbon-free desiccator (i.e. no silicone grease) for 30 min to allow the acid to permeate throughout the sample. The desiccator was then fitted with a Viton seal and evacuated for 2–3 min until equilibrium was reached, then left at this pressure for 1 h to allow HCL to permeate throughout the sample and reduce the likelihood of trapped air. After carefully and slowly returning the desiccator to atmospheric pressure, a further 35 μL of HCL was added, before transferring to a clean oven set to 40 °C for 1 h (or until dry). A final mobilisation of 35 μL de-ionised water was applied. Samples were returned to the oven to dry at 40 °C after which the Ag-foils were closed and analysed by IRMS.

For quantifying inorganic carbon (IC) an excess of hydrochloric acid (7 ml of 4 M HCL) was applied to 5 g of soil and the resulting CO_2 measured using a pressure calcimeter. Soil organic carbon (SOC) was calculated from the difference between total C and IC.

2.6. Stable carbon isotope analysis

The isotopic composition ($\delta^{13}C$) and %C of each sample was measured using IRMS using an Anca SL 20/20 Stable Isotope Analyser (Sercon, UK). The precision of the IRMS for the soil (internal standard) and plant (external standard: wheat flour) material was between 1 and 2% with regard to carbon and <1% for the isotope analysis. The ^{13}C abundance was expressed as delta depletion ($\delta^{13}C‰$) from the international standard, Pee Dee Belemnite (PDB):

$$\delta^{13}C‰ = \left[\frac{R_{sample} - R_{reference}}{R_{reference}}\right] \times 1000 \quad (1)$$

where R_{sample} is the isotope ratio, $^{13}C/^{12}C$, of the sample and $R_{reference}$ is that of PDB.

2.7. Contribution of Miscanthus-derived soil carbon to SOC

The SOC_M fraction of total SOC can be estimated for each soil layer by the balance of the measured $\delta^{13}C$ between the original SOC (SOC_{orig}; reference values) and the signature of C_4 *Miscanthus* inputs.

$$(\delta^{13}C_{meas} \times [SOC_{meas}]) = (\delta^{13}C_{SOC_{orig}} \times [soc_{orig}]) + (\delta \times [SOC_M]) \quad (2)$$

Although a small additive isotope fractionation effect is expected due to heterotrophic microbial respiration depleting SOC of lighter isotopes, in general this appears to be balanced by microbial preference for more ^{13}C enriched substrates (Santruckova et al., 2000; Wynn and Bird, 2007). The fraction of soil carbon derived from *Miscanthus*, F_M, in the ratio of C_4 and total SOC was thus calculated as:

$$F_M = \frac{SOC_M}{SOC_{Tot}} = \frac{(\delta^{13}C_{observed} - \delta^{13}C_{SOC_{orig}})}{(\delta^{13}C_{C4} - \delta^{13}C_{SOC_{orig}})} \quad (3)$$

Table 2
Log-transformed (in brackets) and back-transformed mean RLD's and RDM's for each horizon depth section (A: 0–10, 10–20, 20–30 cm; B: 30–50, 50–70 and 70–100 cm). Statistically significant two-way interactions between the effects of horizon and position with phenotype (A/B vs. NT/T and P/B vs. NT/T, respectively) are presented for RLD, and a three dimensional factor interaction for RDM between horizon, sample position and growth type (A/B × P/G × NT/T), respectively.

		Phenotype NT	Phenotype T	SED	Wald statistic	*p*
RLD transformed mean ($cm\,cm^{-3}$)						
	Horizon A	7.64 (2.03)	5.71 (1.74)	(0.179)	5.18	0.023
	Horizon B	1.25 (0.22)	1.48 (0.39)			
	Position P	3.35 (1.21)	3.84 (1.35)	(0.179)	3.79	0.052
	Position G	2.84 (1.04)	2.20 (0.79)			
RDM transformed mean ($Mg\,ha^{-1}$)						
Position P	Horizon A	2.73 (1.00)	3.01 (1.10)	(0.248)	4.15	0.044
	Horizon B	0.45 (−0.81)	0.44 (−0.83)			
Position G	Horizon A	1.84 (0.61)	1.35 (0.30)			
	Horizon B	0.31 (−1.19)	0.44 (−0.83)			

From this the contribution of *Miscanthus*–derived carbon ($Mg\,C\,ha^{-1}$) was estimated using the profile respective depth and stone-free bulk densities. The $\delta^{13}C$ values of nearby reference arable samples had a mean $\delta^{13}C$ of −28.16‰ (range −28.36 to −27.75‰ PDB) and did not show any systematic variation or statistical significance over depth ($p = 0.29$; $F = 1.46_{4,9}$). A single reference point ($\delta^{13}C = -28.16$) was thus used from which estimates of *Miscanthus*-derived SOC accumulation were estimated. The $\delta^{13}C_4$ marker value for *Miscanthus* used in this study was −11.7‰ $\delta^{13}C$ PDB (close to the −11.8‰ used by Dorodnikov et al. (2007)).

2.8. Statistical analysis

All statistical comparisons were made using GenStat 14 (Payne et al., 2011). Residual maximum likelihood (REML) methods were applied in preference to ANOVA as samples were not equally replicated (2 replicates Sin-H6, Sin-H9; 3 replicates Sin-11, Sac-5, Gig-1). Variables were transformed where necessary after examining the residual diagnostic plots for homogeneity of variance; the natural logarithm was selected as the most appropriate transformation. We present the transformed and back transformed data in Table 2 only for the variables with significant effects between interacting factors; all statistical comparisons were made using the transformed data. Natural means are presented in Figs. 3 and 4.

3. Results

3.1. Root distribution in the profile

Roots of all *Miscanthus* genotypes were up to an order of magnitude more abundant in the A (topsoil) than in the B horizon (subsoil) but varied greatly within each phenotype group (Fig. 1). Statistical significance of the differences between varieties or phenotypes (T vs. NT) was reduced due to spatial variability. Mean log-transformed RLD showed a significant two-way interaction between phenotype and vertical distribution ($p = 0.023$; Table 2). Phenotype 'T' contributed much less RLD to the A horizon but more to the B horizon than NT phenotypes. Additionally, tufted varieties generally showed higher RLD in the plant position than did NT, and less in the gap than NT (Table 2). This interaction was just outside the 5% confidence limit ($p = 0.052$). A 3-way interaction between horizon, position and phenotype was found for root dry matter (RDM; $p = 0.04$), which reflects the strong contrast in spatial distributions in RDM between phenotypes (most notably in the A horizon gap).

Table 3 gives horizon TRL and RDM data on the natural scale. In the A horizon there was typically more than twice the RDM directly under plants (P) of all genotypes except for *M. giganteus*, where RDM appears to be evenly distributed (having equally colonised the gap). However, within the T phenotype the high contrast between allocation of RDM to the G vs. P position of Sin-11 and Sin-H9 ($RDM_{B\%}$) was not seen with Sin-H6 (Table 3). Sin-H6 seems to be an exception to all genotypes (Table 1). Nevertheless, the

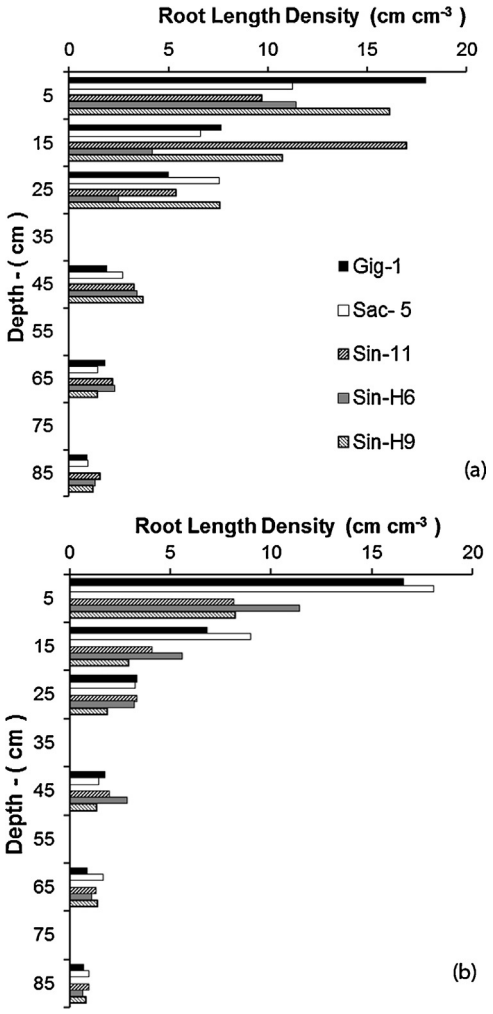

Fig. 1. Back-transformed mean root length density (RLD) under plant (a) and in gap (b). See Table 2 for SED.

Table 3
Total root length (TRL; km m^{-2}), and root dry matter (RDM; Mg ha^{-1}) per horizon (subscript A vs. B), genotype, and sample position (G vs. P).

Genotype	Position	TRL_A (km m^{-2})	TRL_B (km m^{-2})	TRL (km m^{-2})	RDM_A (Mg ha^{-1})	RDM_B (Mg ha^{-1})	$RDM_{B\%}$ (%)
Gig-1 (NT)	P	30.7	10.3	41.0	11.35	3.34	22.8
	G	26.8	7.4	34.2	10.10	2.78	21.6
Sac-5 (NT)	P	25.4	11.2	36.7	7.85	3.30	29.6
	G	30.3	9.0	39.3	4.71	2.17	31.6
Sin-11 (T)	P	32.1	15.8	47.9	14.49	3.93	21.3
	G	15.6	9.5	25.0	4.59	3.43	42.8
Sin-H6 (T)	P	18.1	15.4	33.5	6.88	3.18	31.7
	G	20.2	10.0	30.2	4.38	2.59	37.1
Sin-H9 (T)	P	34.5	14.0	48.5	12.97	4.02	23.7
	G	12.9	7.7	20.7	5.64	4.24	42.9
Phenotype mean							
NT	P			38.8			25.7
	G			36.7			25.1
T	P			44.0			24.0
	G			25.3			41.5

T 'phenotype mean' allocation of RDM to the B horizon ($RDM_{B\%}$) was still nearly double that of NT (41.5% compared to 25.1%; Table 3).

3.2. δ^{13} of soil organic matter (SOM).

The $\delta^{13}C$ signatures in almost all soil samples under *Miscanthus* were less negative than those found in the arable reference profile. *Miscanthus* cover had increased $\delta^{13}C$ up to −25.39 in the B, and −16.37‰ in the A horizons, respectively (Fig. 2). Statistical analysis of $\delta^{13}C$ changes indicated the main effect was depth ($F_{5,78.1} = 178.45$; $p < 0.001$) followed by an interaction between depth and genotype ($F_{20,78.2} = 1.76$; $p = 0.040$). The statistics demonstrate that *Miscanthus* genotype is a relevant factor in SOC distribution over depth. Although the sampling position effect (P vs. G) was found to lie just outside the 5% confidence level ($F_{1,8} = 5.25$; $p = 0.051$) mean $\delta^{13}C$ over depth are presented for samples taken under both the original planting (P) and gap (G) positions, respectively (Fig. 2).

3.3. Changes in SOC_{orig} and SOC_M concentration

The SOC_{orig} concentration in the A horizon under *Miscanthus* had declined to an average of 13.25 g kg^{-1} relative to the reference soil (17.91 g kg^{-1}) but less from what had been measured in the baseline from the archive (16.4 g kg^{-1}; Fig. 3a). In the B horizon, there appears to be little change in the SOC_{orig} (Fig 3b). By comparison with archive SOC values (Fig. 3a) it can be seen that *Miscanthus* more than compensated for losses in SOC_{orig} through inputs of SOC_M. For statistical comparison between genotypes, transformed and back transformed concentration data are presented in Table 4 (similarly in Table 5 for SOC stock change).

3.4. Net change of SOC stocks

Analysis of phenotype (T vs. NT) showed no statistically significant effect on SOC_M stock (Mg ha^{-1}; $p > 0.05$). However, as with $\delta^{13}C$, there was an important interaction between horizon and genotype ($F_{4,14.5} = 2.94$; $p = 0.016$; Table 5). Comparison of back-transformed means show subsoil SOC_M tended to be greatest under Sin-H6 (2.20 Mg ha^{-1}) and lowest under Sin-H9 (0.39 Mg ha^{-1}). Gig-1 showed the greatest residual contribution (back-transformed) to SOC_M in the A horizon (16.64 Mg ha^{-1}) while Sin-11 residual contributions were low (9.84 Mg ha^{-1}).

Determining the change in SOC storage over area is complicated by the large variation of bulk density due to the variation in stone, root, and rhizome content. Mean stone-free bulk densities in the A-horizon directly under the plants were significantly smaller than in the gap (1.10 and 1.40; SED ± 0.077 Mg m^{-3}). The difference was mainly attributable to the T-phenotype, where roots and rhizomes displaced the soil and raised the soil surface. No significant differences were found for the B horizons (1.30 and 1.33 ± 0.077 Mg m^{-3}). Accordingly, statistical analysis of SOC_M concentration (g kg^{-1}) showed a stronger interaction between horizon and

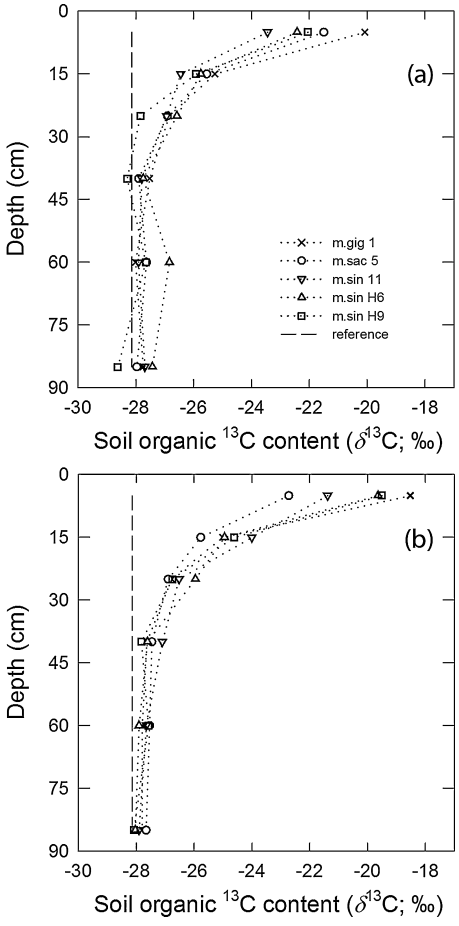

Fig. 2. $\delta^{13}C$ signature of SOM as affected by *Miscanthus* genotype under plant (a) and in gap position (b). Data given on the natural scale (F-statistics for REML are provided in text).

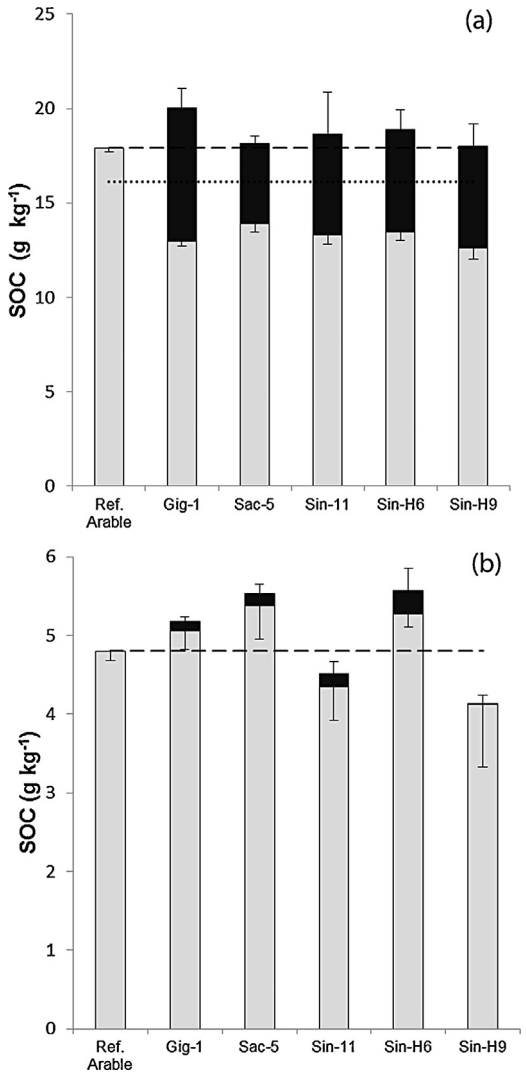

Fig. 3. Mean SOC_{orig} (grey bars) and SOC_M (black bars) concentrations in the A horizon (a) and B horizon (b). Carbon concentration measured in the equivalent soil mass (ESM) corresponding to 0–30 cm for *Miscanthus* and 0–26 cm for Reference Arable. Depths of B horizons correspond to 30–100 cm for *Miscanthus* and to 26–96 cm for Reference Arable. SOC in Reference Arable (- - -) and archive (· · · · · ·); archive SOC for B horizon not available. Positive bars indicate standard error for $SOC_{M,}$ negative bars for SOC_{orig}.

genotype ($F_{4,13.5} = 7.46$; $p = 0.002$; Table 4) than estimates over area (Table 5). Again however, there was no statistically significant effect of phenotype (growth-form) upon SOC stocks.

Fig. 4a and b show natural mean stock estimates over area for SOC_{orig} and SOC_M combined (inclusive of bulk density effects). Due to high residual variability there were no statistically significant differences in transformed SOC_{orig} data (for statistical comparison

Table 4
Log-transformed (in brackets) and back-transformed mean SOC_M concentrations by horizon (g kg^{-1}). SOC_M distribution was affected by a statistically significant two-way interaction between genotype and horizon ($F_{4,13.5} = 7.46$; $p = 0.002$).

Genotype	Phenotype class	SOC_M (g kg^{-1})	
		Horizon A	Horizon B
Gig-1	NT	(1.89) 6.63	(−2.19) 0.11
Sac-5	NT	(1.41) 4.09	(−1.72) 0.18
Sin-11	T	(1.37) 3.92	(−1.80) 0.17
Sin-H6	T	(1.64) 5.17	(−1.10) 0.33
Sin-H9	T	(1.59) 4.92	(−3.31) 0.04
s.e.d		(0.387)	

Table 5
Log-transformed (in brackets) and back-transformed SOC_M stock in horizon A vs. B (per unit area). SOC_M distribution was affected by a statistically significant two-way interaction between genotype and horizon ($F_{4,14.5} = 4.40$; $p = 0.016$).

Genotype	Phenotype class	SOC_M (Mg ha^{-1})	
		Horizon A	Horizon B
Gig-1	NT	(2.81) 16.64	(−0.07) 0.93
Sac-5	NT	(2.59) 13.26	(0.39) 1.48
Sin-11	T	(2.29) 9.84	(0.39) 1.48
Sin-H6	T	(2.74) 15.52	(0.79) 2.20
Sin-H9	T	(2.75) 15.71	(−0.93) 0.39
s.e.d		(0.364)	

of SOC_M between genotypes see Table 5). Strictly speaking, no statistical calculation can be applied for the stock change in SOC_{orig} by reference to the arable plot, as the reference arable is not within the randomised block design. However, *Miscanthus* inputs (SOC_M) appeared to compensate for losses in SOC_{orig} in all cases except with Sin-11 (Fig. 4a). This was not seen on a concentration basis

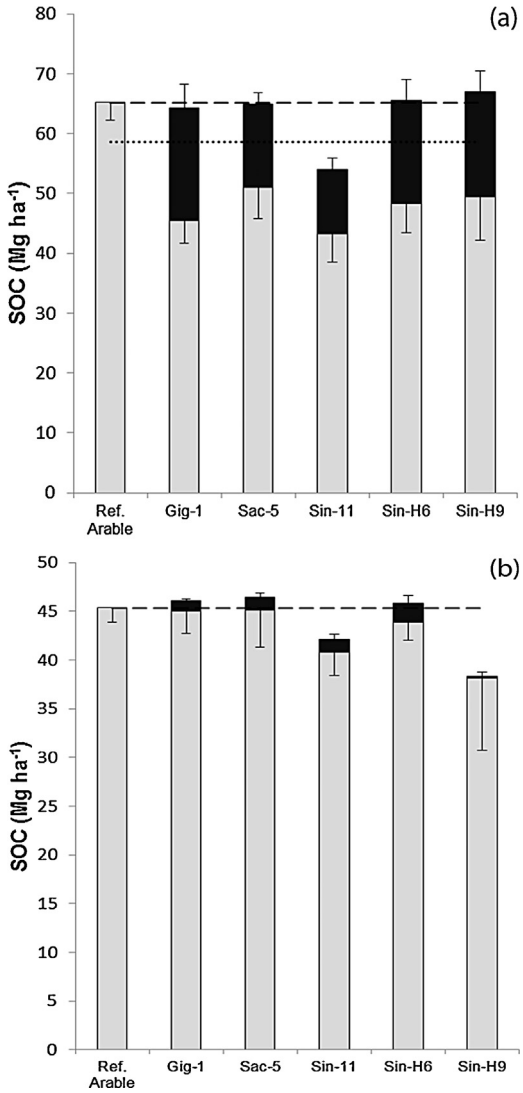

Fig. 4. Total SOC_{orig} (grey bars) and SOC_M (black bars) stocks in the A horizon (a), and B horizon (b). Carbon stock estimated on the basis of equivalent soil mass (ESM) corresponding to 0–30 cm for *Miscanthus* and 0–26 cm for Reference Arable. Depths of B horizons correspond to 30–100 cm for *Miscanthus* and to 26–96 cm for Reference Arable. SOC in Reference Arable (- - -) and archive (· · · · · ·); archive SOC for B horizon not available. Positive bars indicate standard error for $SOC_{M,}$ negative bars for SOC_{orig}.

(Fig. 3a), indicating that the A horizon of Sin-11 had a lower average bulk density. Comparison indeed showed its bulk density to be the lowest among all genotypes (1.12 Mg m^{-3} vs. 1.19, 1.23, 1.20 and 1.35 Mg m^{-3} for Gig-1, Sac-5, Sin-H6 and Sin-H9, respectively).

4. Discussion

The results presented describe root distribution for a range of fundamentally different *Miscanthus* genotypes of the two major phenotypes (T/NT-growth forms) and the impact of their long-term cultivation on the SOC of a silty clay loam in England. Similar experiments exist in Germany (Gauder et al., 2012) and Denmark (Jorgensen et al., 2003) but from those studies no data for roots and *Miscanthus*-derived SOC have been published. Our data for RLD, root biomass, and their distribution down the profile are consistent with those reported for *M.* × *giganteus*, the only commercially grown genotype (Neukirchen et al., 1999; Amougou et al., 2011; Monti and Zatta, 2009; Dohleman et al., 2012). Our research illustrates the spatial heterogeneity (horizontal and vertical) of rooting and carbon allocation. Analysis also shows that SOC enrichment is more closely correlated with RLD than root biomass, which is consistent with findings that link carbon inputs to root exudates and rhizo-depositions from finer roots (Kanova et al., 2010; Techer et al., 2011).

4.1. Is a certain rooting pattern a desirable trait?

The similarity in RDM distribution irrespective of position with *M.* × *giganteus* (Table 3) suggests that the mature stand had completely colonised the A horizon (only a 13% difference between horizontal sampling points 32.5 cm apart). For a young *M.* × *giganteus* stand RLD is considerably lower in the gap between plants than in the plant center (Neukirchen et al., 1999). A recent comparison of different *M. sacchariflorous* × *M. sinensis* crosses found genotypes that spread their root system less than *M.* × *giganteus* and exhibited a growth form that was still very much akin to the T phenotype (Zatta et al., 2014). However, their data do not distinguish between root and rhizome fractions of the BGB, although RDM traits could be approximated from biomass in their deeper horizon (15–30 cm).

RDM allocation to the subsoil (B horizon) is thought to be a desirable trait (Kell, 2011) but rarely studied as sampling poses a challenge in terms of temporal and spatial variability (Neukirchen et al., 1999). Here, C allocation to the B horizon was found to be lower in the NT than in the T phenotype (25 vs. 33%), presumably because of the high allocation of C into rhizomes and associated roots. T types spread laterally to the B horizon in the gap (Table 3). Spatial variability between replicates was large, and RLD did not show a statistically significant interaction between phenotype and sampling position whilst it exists for RDM (Table 2).

The T phenotypes included genotypes with contrasting AGB traits which could be mirrored in BGB accumulation. Sin-11 and Sin-H9 showed high proportional allocation of RDM to the B horizon (>40%), whilst Sin-H6 did not follow the same pattern (Table 3; neither for RDM_A nor RDM_B). Litter accumulation was also low for this genotype (Table 1) but a causal link between these observations for Sin-H6 is unknown. The concomitant high SOC_M and low residual litter, however, suggest potentially high SOC accumulation from more rapidly decomposing leaf and root litter. Parameters of *Miscanthus* residues differ (see turnover of root and leaf litter; Amougou et al., 2011) but our results indicate a wide range for genotype-specific parameters. The relationship between RLD and *Miscanthus*-derived SOC (Fig. 5) identifies genotype Sin-H6 as an example showing greater SOC_M accumulation per unit root length. This supports the hypothesis that faster turnover of roots and litter increase SOC_M. It would therefore be of great interest to characterise the biochemical composition of roots and leaf residues to explore the reasons behind the observed contrast.

The genotype-specific variation of carbon allocation was previously observed in controlled conditions (Clifton-Brown et al., 2001). Data from the field are the products of greater functional complexity of C turnover, affected by temperature (Beuch et al., 2000; Magid et al., 2004; Amougou et al., 2011) and soil hydrology, especially in the A horizon. The contrasting carbon allocation at depth for Sin-11 and Sin-H6 to the B horizon (Table 4) could reflect the different responses to water stress shown in laboratory experiments: In contrast to *M.* × *giganteus* and *M. sacchariflorus*, the *M. sinensis* type (Clifton-Brown and Lewandowski, 2000) showed higher drought-tolerance possibly due to increased RLD at depth.

The high proportional RDM allocation to the B horizon and lateral spread under Sin-11 (Table 3) suggest that root density was the primary factor influencing the accumulation of SOC_M (1.48 Mg ha^{-1}; Table 4). In contrast, Sin-H9 resulted in the lowest quantity of SOC_M in spite of high absolute and relative RDM to the B horizon. The fact that there was a statistically significant interaction of SOC_M allocation to soil horizon with individual genotype (Table 4), but not growth form (T vs. NT) indicates that other factors affect the SOC_M distribution between soil profiles. Production of root exudates and other rhizo-depositions can be of similar magnitude as roots (Rasse et al., 2005; Techer et al., 2011). These root-associated biochemical factors may explain the differences between the effects of roots on SOC_M and SOC_{orig} in the B horizon for genotypes Sin-11 and Sin-H9. Priming of existing SOM in response to the input of easily decomposable organic substrate is a transient phenomenon (Kuzyakov, 2002). These potentially accelerate the degradation of SOC_{orig} as observed for *Miscanthus* phenotypes (Figs. 3 and 4) as suggested by Zatta et al. (2014). In this context it is controversial how the addition of mineral N affects SOC turnover (e.g. Wang et al., 2014; Foereid et al., 2004; Amougou et al., 2011). The differences we found for the impact of RLD on SOC_M under different genotypes need further research, also with regard to the degradation of SOC_{orig}.

4.2. Miscanthus effects on carbon stocks

The comparison of SOC over area (Table 5; Mg ha^{-1}) is affected by the change in bulk density (ρ_{SF}) over time. The change in ρ_{SF} affects sampling depth because soil cores taken to a fixed depth will not access the same mineral soil as before expansion (Ellert and Bettany, 1995). Such artefacts have been discussed by

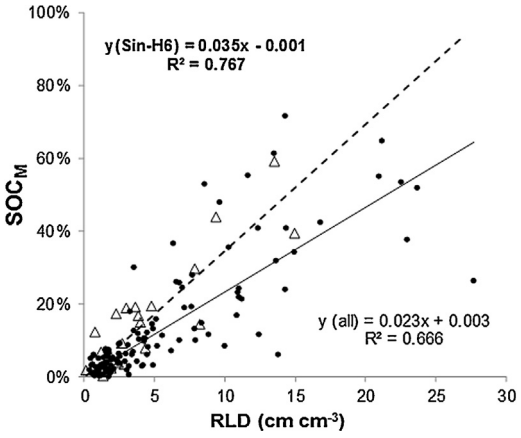

Fig. 5. *Miscanthus* derived soil carbon (SOC_M) as % of total SOC versus root length density (RLD) under various *Miscanthus* genotypes; "△" Sin-H6, "●" all genotypes.

Palm et al. (2014) in the context of tillage effects on soil C stocks, proposing Equivalent Soil Mass (ESM) sampling. In practical terms, an ESM controlled sampling strategy is only possible once the bulk density has been measured; however, pre-emptive sampling of every plot would be prohibitively expensive.

In the present study the SOC quantities over area incorporate variation of ρ_{SF}, thus, we also compared SOC change on a concentration basis (g kg^{-1}; Table 4 and Fig. 3). In this way, plot variability in ρ_{SF} was avoided and genotype-related effects were identified with greater statistical confidence, at $p = 0.002$ (Table 4) as opposed to $p = 0.016$ (Table 5). Measurement on a concentration basis also provided more precise estimates of inputs (Fig. 3) as anticipated by Lee et al. (2009). Figs. 3a and 4a show that, while SOC_{orig} declines over 14 years of *Miscanthus* cropping from its baseline (archive sample), the total SOC increased (~5 Mg ha^{-1}) above the baseline and was, in spite of much lower N inputs, similar to the high N reference arable. In contrast, any SOC_{orig} decline or net SOC increase in the B horizon (Figs. 3b and 4b) was not measurable. This is in accordance with the premise that deep C is more stable due to closer organo-mineral interactions (Schrumpf et al., 2013).

Three distinct groups of *Miscanthus* can be separated according to their effects on the A horizon (Fig. 3a): (1) *M. giganteus,* which contributes the greatest SOC_M with accompanied greatest loss of SOC_{orig}; (2) *M. sacchariflorus* with lowest contributions of SOC_M and greatest retention of C_3 originating SOC; (3) *M. sinensis* with intermediate and similar effects on respective SOC concentrations. Recent analysis showed that the average fertiliser application of 50 kg N ha^{-1} yr^{-1} as ammonium nitrate to *M.* × *giganteus,* applied in the present study, would limit biomass production (Shield et al., 2014). This would be even more relevant for *M. sinensis* genotypes as these have only a very small rhizome system (Table 1), which would limit recycling of N within the plant (Amougou et al., 2011). It is hypothesised here that under conditions of N limitation the production of labile *Miscanthus* root exudates increases to support a microbial community capable of mineralising soil organic N and thus depleting SOC. Similar ideas have been discussed by Kuzyakov (2002) but empirical studies are now needed to investigate this and quantify its potential impact upon calculations of energy budgets for carbon crops.

4.3. Meta-contributions to SOC sequestration

SOC_{orig} varied greatly between plots and genotypes and no statistically significant differences were observed. However, any differences in expansion of the soil profile due to rhizome and root growth will physically protect SOC. Lower temperature and oxygen, protection from freeze-thaw and drying and wetting will all affect microbial activity and contribute to the preservation of SOC (Balesdent et al., 2000). Sequestration of C in soil purely through burying is a commonly overlooked mechanism (Chaopricha and Marin-Spiotta, 2014). In temporal perennials its sustainability will depend on the form of reversion to arable. Nevertheless, the expanding surface horizons (burying subsoil) could be a useful indirect trait to develop otherwise shallow soils on marginal land and compliment the established benefits from protection against soil erosion (e.g. Kroumov and Blagoeva, 2011). Interestingly, the SOC_{orig} 'burying effect' could be of potentially greater contribution to sequestration of C than the direct allocation of *Miscanthus* C at depth because SOC_{orig} is likely to be more closely associated with the mineral fraction and more stable than recent C inputs in subsoils (Schrumpf et al., 2013). Furthermore, turnover rates of SOC are inversely related to concentration (Don et al., 2013) which is inherently low in the subsoil. The apparent uncertainty about changes in SOC_{orig} in the subsoil (Figs. 3b and 4b) demand further evidence to support these concepts.

5. Conclusions

We found variation in root distribution between genotypes, with 'T-phenotype' (tuft growing genotypes) allocating more biomass – relatively (33 vs. 25%) and absolutely (3.6 vs. 2.9 Mg ha^{-1}) – to roots at greater depth than NT phenotypes. Analysis of SOC concentration and isotope composition revealed allocation patterns for SOC_M to be significantly different with respect to depth and genotype (Table 4). Within the T phenotype, the higher dry matter allocation and lateral spread of roots in the subsoil observed for Sin-11 and Sin-H9 (Table 3) were not seen with Sin-H6, indicating high diversity within this phenotype.

These results reveal a statistically significant link between RLD distribution and newly derived SOC_M which supports the premise that, for C sequestration, it is important to consider the effect of all carbon inputs, including short-lived rhizo-depositions. Furthermore, a subgroup of higher SOC_M values associated with low root volumes in *M. sinensis* could point to higher root turnover in some species (Sin-H6), a hypothesis to be followed up in research that integrates N and C turnover.

In view of the limiting quantity of N-fertiliser, the accumulation of SOC_M in this trial is likely to be lower than would occur under optimum conditions for biomass production. The net SOC stock increase under low N input (50 kg N ha^{-1} yr^{-1}) *Miscanthus* production (<10 Mg ha^{-1} yr^{-1}) was small (<0.5 Mg SOC ha^{-1} yr^{-1}). Although its C stock was similar to the SOC in a high input arable reference soil after 14 years of cultivation, its net carbon gain (CO_2 eq.) is by far superior when accounting for the respective average N fertiliser inputs (50 vs. 141 kg N ha^{-1}).

Future work could expand on the contribution of root exudates and (D)OC from the litter to SOC_M and whether the effects of chemical and physical properties on decomposition can be disentangled (texture and aggregation of the soil associated with higher SOC_M/root ratios). The measured SOC_M enrichment and SOC_{orig} decline as well as biomass production, litter and root accumulation will be instrumental in the calibration and validation of models such as RothC for simulating soil C sequestration under *Miscanthus*.

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