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Gregory, A. S., Dungait, J. A. J., Shield, I. F., Macalpine, W. J., Cunniff, J., Durenkamp, M., White, R. P., Joynes, A. and Richter, G. M. 2018. Species and Genotype Effects of Bioenergy Crops on Root Production, Carbon and Nitrogen in Temperate Agricultural Soil. *BioEnergy Research*. 11 (2), pp. 382-397.

The publisher's version can be accessed at:

- <https://dx.doi.org/10.1007/s12155-018-9903-6>

The output can be accessed at: <https://repository.rothamsted.ac.uk/item/84735/species-and-genotype-effects-of-bioenergy-crops-on-root-production-carbon-and-nitrogen-in-temperate-agricultural-soil>.

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# Species and Genotype Effects of Bioenergy Crops on Root Production, Carbon and Nitrogen in Temperate Agricultural Soil

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Published online: 20 March 2018  
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## Abstract

Bioenergy crops have a secondary benefit if they increase soil organic C (SOC) stocks through capture and allocation below-ground. The effects of four genotypes of short-rotation coppice willow (*Salix* spp., ‘Terra Nova’ and ‘Tora’) and *Miscanthus* (*M. × giganteus* (‘Giganteus’) and *M. sinensis* (‘Sinensis’)) on roots, SOC and total nitrogen (TN) were quantified to test whether below-ground biomass controls SOC and TN dynamics. Soil cores were collected under (‘plant’) and between plants (‘gap’) in a field experiment on a temperate agricultural silty clay loam after 4 and 6 years’ management. Root density was greater under *Miscanthus* for plant (up to 15.5 kg m<sup>-3</sup>) compared with gap (up to 2.7 kg m<sup>-3</sup>), whereas willow had lower densities (up to 3.7 kg m<sup>-3</sup>). Over 2 years, SOC increased below 0.2 m depth from 7.1 to 8.5 kg m<sup>-3</sup> and was greatest under Sinensis at 0–0.1 m depth (24.8 kg m<sup>-3</sup>). *Miscanthus*-derived SOC, based on stable isotope analysis, was greater under plant (11.6 kg m<sup>-3</sup>) than gap (3.1 kg m<sup>-3</sup>) for Sinensis. Estimated SOC stock change rates over the 2-year period to 1-m depth were 6.4 for Terra Nova, 7.4 for Tora, 3.1 for Giganteus and 8.8 Mg ha<sup>-1</sup> year<sup>-1</sup> for Sinensis. Rates of change of TN were much less. That SOC matched root mass down the profile, particularly under *Miscanthus*, indicated that perennial root systems are an important contributor. Willow and *Miscanthus* offer both biomass production and C sequestration when planted in arable soil.

**Keywords** Carbon-13 isotope · Carbon sequestration · Land use change · *Miscanthus* · Perennial bioenergy crop · *Salix*

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s12155-018-9903-6>) contains supplementary material, which is available to authorized users.

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## Introduction

There has been an increase in the use of dedicated biomass crops to exploit photosynthesis for bioenergy production over recent decades to address two pressing global concerns: C emission reduction and energy security [1, 2]. Two dedicated low-input bioenergy crops frequently planted in temperate regions, such as the UK, are willow (*Salix* spp.) in short rotation coppice (SRC) systems and species of the perennial grass genus *Miscanthus* [3]. Commercial willow plantations produce 9–12 Mg ha<sup>-1</sup> year<sup>-1</sup> of biomass in 2–4-year SRC harvest rotations typically [1, 4–6]. *Miscanthus* is an annually-harvested perennial rhizomatous grass originating from Asia which has C<sub>4</sub> physiology and can produce biomass yields of 12–15 Mg ha<sup>-1</sup> year<sup>-1</sup> in the UK [1, 2, 6, 7]. In England in 2015, there were 2885 ha under SRC (willow and *Populus*), yielding 17–35 Gg of dry biomass, of which 15 Gg was used in power stations, and 6905 ha under *Miscanthus*, yielding 69–104 Gg of dry biomass, of which 33 Gg was used in power stations [6].

In addition to production of biomass, perennial bioenergy crops may have a secondary benefit if they increase stocks of soil organic C (SOC), and total N (TN) by association, through capture of atmospheric C and allocation to below-ground plant biomass [8]. Realising the potential for C sequestration over the lifetime of the stand, which is typically more than 15 years and up to 30 years for willow [9, 10], is likely as perennial bioenergy crops require no cultivation during their lifetime, aside from at planting, and hence disturbance to the soil and the root system is minimised. The roots may persist longer than under annual crops, which is important knowing that SOC is primarily derived from roots [8, 11]. The roots do need to turn over, however, to be incorporated into SOC, operationally defined as the < 2 mm soil size fraction, in order to contribute to sequestration. Perennial bioenergy crops allocate nutrients and C below ground to the root system during senescence in readiness for the following growing season [1, 12], and some crops extend their root systems deep into the subsoil, especially *Miscanthus* [8, 13, 14]. In their comprehensive review, Agostini et al. [15] found that root C stocks as an input were greater for willow (1.0 Mg ha<sup>-1</sup> year<sup>-1</sup>) than *Miscanthus* (0.5 Mg ha<sup>-1</sup> year<sup>-1</sup>), but that the latter had a greater mean residence time (1.3 years) than the former (1.8 years), based on the finer nature of willow roots and their fast turnover. The importance of root inputs to SOC is likely dependent on their physical (diameter, association with soil structure) and chemical (greater or lesser ‘labile’ compounds) characteristics.

The potential for C sequestration is site-specific in part, being dependent on local environmental and management factors, including previous land use which largely controls initial SOC and TN contents [2, 8, 16–18]. Whilst it may be desirable that bioenergy crops are concentrated on less-productive ‘marginal’ land [8, 16], land used for food production has also been planted with bioenergy crops, causing a conflict between food and bioenergy production on higher-quality soils [2]. This is important because whether such crops are established on degraded or fertile soils may control the potential for C sequestration [8]. Also important is the age of the stand, as others have reported an establishment phase as crop yields increase [19] where resident SOC turns over before full replacement by new SOC deriving from the bioenergy crop [16], particularly where the former land use was under perennial grass [18, 20, 21]. Therefore, a full assessment of the potential for bioenergy crops to sequester significant amounts of C is still far from certain [8, 15, 22, 23] and is reliant on monitoring the dynamics of SOC in well-designed field experiments. It has been estimated that sequestration under bioenergy crops needs to be at least 0.25 Mg SOC ha<sup>-1</sup> year<sup>-1</sup> for the system to be truly C-neutral [15, 24].

We sought to assess the effect of different species and genotypes of bioenergy crops on root production, SOC and TN in temperate agricultural soil. With detailed prior knowledge of

species and genotypic differences in above-ground traits of such bioenergy crops, the main hypothesis was that differences would also be reflected in the below-ground biomass and that these, in turn, affect SOC and TN dynamics. An existing field experiment in the UK with established stands of different willow varieties (genotypes) and *Miscanthus* genotypes, the principal crops grown solely for bioenergy production in the UK, was used to test the hypothesis. Below-ground biomass and quantified SOC and TN were compared in the underlying soil on two successive occasions when the bioenergy crop stand was 4 and 6 years old to assess stocks and changes. In addition to bulk SOC, the natural abundance <sup>13</sup>C isotope labelling of *Miscanthus*-derived SOC was used to estimate new C input in the establishment phase (inside 10 years).

## Materials and Methods

### Field Experiments and Treatments under Study

We focused on a field experiment established in 2009 at Rothamsted Research (Hertfordshire, UK) as part of the UK Biotechnology and Biological Sciences Research Council, Sustainable Bioenergy Centre [5]. Four genotypes each of willow and *Miscanthus* were planted in plots in a randomised block design with four replicates on a field previously under annual arable crops for at least 50 years [25]. Planting followed conventional commercial best practice [9, 10]: willows were planted as cuttings using the typical twin-row design at a planting density of 16,667 plants ha<sup>-1</sup> and *Miscanthus* was planted in single rows at a planting density of 20,000 plants ha<sup>-1</sup>. Willows were cut back at the end of the establishment year in early 2010 and then subjected to a 2-year SRC regime thereafter, whereas *Miscanthus* was harvested annually. Harvesting and coppicing were done in January. *Miscanthus* plots received 100 kg N ha<sup>-1</sup> every year in May and willow plots received 60 kg N ha<sup>-1</sup> after each 2-year harvest (May 2010, 2012 and 2014), both as Nitram® (NH<sub>4</sub>NO<sub>3</sub>; 34.5% N), following typical guidelines during the establishment phase [9, 10]. Canopy traits were used to select two of the four willow varieties for investigation. ‘Terra Nova’ has short, ovate leaves, with a plant leaf area of 1.4 m<sup>2</sup> per plant [5] and an average leaf area index of 1.93 [4], whereas ‘Tora’ has long, lanceolate leaves with a plant leaf area of 0.8 m<sup>2</sup> per plant [5] and an average leaf area index of 1.26 [4]. Similarly, we chose two standard *Miscanthus* genotypes: *M. × giganteus* Greef et Deu ex Hodkinson & Renvoize is a non-tufted tall-growing genotype, and *M. sinensis* Andersson is a tufted genotype with a shorter stature and which produces many more, thinner stems. Details of the site, soils and experiment [26–29] are given in Table 1, and full details of characteristics of the genotypes are given elsewhere (Supplementary Material; [5, 23, 30]). For brevity, the genotypes are termed Terra Nova, Tora, Giganteus and Sinensis hereafter.

**Table 1** Details of the site, soil and experiment

Site	Rothamsted Research		
Location	Harpenden, Hertfordshire, UK		
Global position (latitude; longitude) (°)	51.816; −0.381		
Altitude (GB Ordnance Datum) (m)	132		
Maximum annual temperature (°C) <sup>a</sup>	13.6		
Minimum annual temperature (°C) <sup>a</sup>	6.0		
Mean annual rainfall (mm) <sup>a</sup>	733		
Soil type (SSEW Soil Group) <sup>b</sup>	Paleo-argillic brown earth		
Soil type (WRB Reference Soil Group) <sup>c</sup>	Haplic Luvisol		
Soil			
Depth (m)	0.00–0.23	0.23–0.60	0.60–0.90
Horizon <sup>b</sup>	Ap	Bt	Bc(g)
Sand (g kg <sup>−1</sup> )	177	124	105
Silt (g kg <sup>−1</sup> )	618	604	630
Clay (g kg <sup>−1</sup> )	205	272	265
Soil texture <sup>b</sup>	Silty clay loam		
pH (soil/water, 1:2.5) (−log <sub>10</sub> (g[H <sup>+</sup> ] L <sup>−1</sup> ))	7.35	7.54	7.68
Organic C (g kg <sup>−1</sup> )	13.10	6.11	3.48
Total N (g kg <sup>−1</sup> )	1.39	0.76	0.52
NH <sub>4</sub> (mg kg <sup>−1</sup> )	2.71	1.21	0.47
NO <sub>3</sub> (mg kg <sup>−1</sup> )	2.62	1.10	0.66
PO <sub>4</sub> -P (mg kg <sup>−1</sup> )	27.74	7.16	4.61
Bulk density (Mg m <sup>−3</sup> )	1.47	1.48	1.61
Experiment	BSBEC trial <sup>d</sup>		
Previous land use	Cereal crops		
Experiment planted	2009		
Experimental design	Randomised complete block		
Field replication	4		
Plant	Willow	<i>Miscanthus</i>	
Genotypes	4	4	
Plots	16	16	
Plot size (m)	26.4 × 8.5	7.2 × 8.5	
Plant spacing between rows/twin rows (m) <sup>e</sup>	1.60	0.65	
Plant spacing within twin rows (m) <sup>f</sup>	0.80	–	
Plant spacing within rows (m)	0.50	0.77	
Planting density (plants ha <sup>−1</sup> )	16,667	20,000	
Age of shoots at sampling (months)	17	4	
Terms used to describe genotypes sampled	Terra Nova	Giganteus	
	Tora	Sinensis	

<sup>a</sup> For the period 1981–2010

<sup>b</sup> Soil Survey of England and Wales

<sup>c</sup> World Reference Base for Soil Resources

<sup>d</sup> UK Biotechnology and Biological Sciences Research Council, Sustainable Bioenergy Centre (BSBEC) project

<sup>e</sup> This is the *gap* location referred to in this work (see ‘[Materials and Methods](#)’ section for further details)

<sup>f</sup> Only willow was planted in double rows

## Sample Collection and Preparation

We took intact soil cores (0.07 m diameter × 1 m length) using a steel core containing an inner sleeve which was driven into the soil using a hydraulic hammer and extracted using a tripod

ratchet. As the crops were planted in rows (Table 1), two cores were collected from each plot containing the four genotypes: one in the twin row as close to the plant as possible (willow) or directly over the plant (*Miscanthus*), and another in the gap equidistant between rows (or twin rows for willow) of plants.

This sampling regime was adopted to capture the spatial pattern associated with plants located at regular distances and are termed ‘plant’ and ‘gap’ locations hereafter. The cores were collected in the early summer when the stand age was 4 (June 2013) and 6 years (June 2015). In all, therefore, we collected 64 cores (4 genotypes  $\times$  2 locations (plant and gap)  $\times$  4 replicate plots (blocks)  $\times$  2 years).

All cores were wrapped in polythene to keep them intact and stored at  $-18\text{ }^{\circ}\text{C}$  immediately after collection. In the laboratory, each core was brought to room temperature and divided into five depth interval samples: 0–0.1, 0.1–0.2, 0.2–0.3, 0.3–0.5 and 0.5–1.0 m. Each depth interval sample was then further divided in half longitudinally and weighed fresh. For one of the half-interval samples, we washed the soil away gently to leave the  $> 2\text{ mm}$  fraction (stones, litter and rhizome) for mass ( $105\text{ }^{\circ}\text{C}$  (stone) or  $80\text{ }^{\circ}\text{C}$  (plant) for 48 h) and volume adjustment, and the washed roots which were stored in water at  $4\text{ }^{\circ}\text{C}$  prior to analysis. The other half-interval sample was air-dried and crumbled to yield the same  $> 2\text{ mm}$  fraction for mass and volume adjustment, and the soil fraction ( $< 2\text{ mm}$ ). A soil subsample was milled to  $< 350\text{ }\mu\text{m}$  with a Retsch PM 400 planetary ball mill (Retsch GmbH, Haan, Germany) for analysis and a separate subsample was oven-dried at  $105\text{ }^{\circ}\text{C}$  for 48 h to calculate water contents (air-dried and field-moist) and dry mass. We estimated the linear compaction introduced to the soil core during sampling through comparing the length of the soil core to the depth of the hole from where it had been taken, to fully ‘reconstruct’ the core in its original compaction-free field state. We encountered negligible compaction (mean = 1.1%) as the cores were taken when the soil was not in a plastic state.

To provide a baseline from which to compare, we made use of an existing data set collected before the field experiment was established. In July 2009, 16 soil samples were collected to a depth of 0.9 m using a 0.02-m-diameter hydraulic Norsk Hydro Soil Sampler (Norsk Hydro ASA, Oslo, Norway) from locations covering the area of the field experiment. The samples were divided into three depths (0–0.23, 0.23–0.60 and 0.60–0.90 m) to measure various soil properties, including SOC and TN concentration (see method below). In October 2009, small intact soil cores (0.077 m diameter  $\times$  0.05 m length) were collected from ten depths in the top 1 m at four random locations in the field to measure soil dry bulk density. These data were adjusted to the same three depths as above pro rata. The baseline data set is given in Table 1.

## Root Analysis

We subjected washed roots to image analysis using the WinRHIZO 2008a program (Regent Instruments Inc., Québec, Canada) connected to an EPSON Expression 1600 3.4 (Epson America Inc., Long Beach, CA, USA) scanner. Roots were spread onto an A4 scanner bed, covered with a

layer of water and scanned. The resulting binary image was analysed to determine the mean root diameter and the root length density (RLD; length per volume of soil). The roots were dried at  $80\text{ }^{\circ}\text{C}$  for 48 h to calculate their gravimetric concentration (per mass of soil) and the volumetric density (per volume of soil) through knowing the soil bulk density:

$$root_{\text{density}} = root_{\text{concentration}} \cdot \rho_b \quad (1)$$

where  $root_{\text{density}}$  is the root density ( $\text{kg m}^{-3}$ ),  $root_{\text{concentration}}$  is the root concentration ( $\text{g kg}^{-1}$ ) and  $\rho_b$  is the soil dry bulk density ( $< 2\text{ mm}$  soil mass, total volume;  $\text{Mg m}^{-3}$ ).

## Soil Analysis

Soil was analysed for total C and TN concentration using a Leco TruMac Combustion Analyser (LECO Corp., St Joseph, MI, USA). Inorganic C was analysed using a Skalar Primacs AIC Analyser (Skalar Analytical BV, Breda, Netherlands) and the difference with total C yielded the SOC concentration. Inorganic C was only a very minor component of total C (mean  $0.13\text{ g kg}^{-1}$ ,  $n = 320$ ) at the experimental site. Volumetric densities of SOC and TN were then calculated using the same approach as above (Eq. 1) with the same units (replacing ‘root’ with ‘SOC’ or ‘TN’). Following others [18, 31], we used an ‘equivalent soil mass’ approach to adjust the measured SOC and TN density of the initial baseline data to that based on the bulk density measured after 4 and 6 years, which did not differ significantly. For only the soils collected from *Miscanthus* plots, the  $^{13}\text{C}/^{12}\text{C}$  stable isotope ratios were quantified on prepared carbonate-free soil [23] with an IsoPrime 100 Stable Isotope Ratio Mass Spectrometer (Isoprime Ltd., Cheadle Hulme, UK) coupled with a Vario MICRO Cube Elemental Analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). By convention, the  $^{13}\text{C}/^{12}\text{C}$  ratio was expressed as a  $\delta$  value (‰) relative to the international Vienna Pee Dee Belemnite (VPDB) standard. As *Miscanthus* has  $\text{C}_4$  physiology, we used the bulk soil  $\delta^{13}\text{C}$  value to estimate the SOC density derived from *Miscanthus* compared to older SOC deriving from the previous land use (all arable crops with  $\text{C}_3$  physiology):

$$SOC_{\text{density-M}} = \left( \frac{\delta^{13}\text{C} - \delta^{13}\text{C}_{\text{C}_3}}{\delta^{13}\text{C}_M - \delta^{13}\text{C}_{\text{C}_3}} \right) \cdot SOC_{\text{density}} \quad (2)$$

where  $density-M$  indicates the *Miscanthus*-derived SOC density ( $\text{kg m}^{-3}$ ),  $\delta^{13}\text{C}_M$  is the reference  $\delta^{13}\text{C}$  value representative of *Miscanthus* shoot and root plant material ( $-11.70\text{ }‰$ ), and  $\delta^{13}\text{C}_{\text{C}_3}$  is the reference  $\delta^{13}\text{C}$  value representative of the soil at the study site under the previous  $\text{C}_3$  (cereal crops) plants ( $-28.16\text{ }‰$ ) [23].



We estimated area-based SOC and TN stocks at the larger scale (field or plantation) by a three-stage calculation. Firstly, we calculated a revised density using Eq. 1 but with a mean bulk density for each genotype  $\times$  location  $\times$  depth treatment (this was more appropriate when upscaling rather than individual replicate bulk densities) and then multiplied this by the depth interval of interest (m), to give an area-based stock ( $\text{kg m}^{-2}$ ). Secondly, we combined intervals to make larger 0–0.3 m ('topsoil') and 0.3–1.0 m ('subsoil') depth stocks. Finally, we estimated the proportion of land that is most influenced by the plant, for which the *plant* stock is most representative, and the remainder, which is represented by the *gap* stock. For Terra Nova, Tora and Giganteus, stocks were similar for both *plant* and *gap* (see later), but nevertheless we used the minimum distance in the field between adjacent plants (0.50 m for willow in a row and 0.65 m for Giganteus between rows; Table 1), and assumed that each plant exerted a circular influence on the soil from its centre to the distance halfway to its nearest neighbour. Thus, we assumed the circular *plant* radius was 0.25 m for both willows and 0.33 m for Giganteus. For Sinensis, which had a well-defined tuft, the tuft circumference was measured on four representative plants on all four plots in August 2014 and October 2015 (representative of the stand at 4 and 6 years) and the mean radii were 0.17 m in 2014 and 0.18 m in 2015. Using these radii with the planting density of each genotype (Table 1), we were able to divide the total area into *plant* and *gap* and hence adjust the stock estimates pro rata. These stocks, and the estimated initial baseline stock, were analysed together with the rate of change between 0, 4 and 6 years, as expressed in conventional units ( $\text{Mg ha}^{-1}$ ).

## Statistical Analysis

All statistical analysis was performed using the GenStat (18th edition) program (VSN International Ltd., Hemel Hempstead, UK). We transformed the soil and root variates by  $\log_{10}$  firstly to normalise the distribution of the residuals. We then analysed the variates using residual maximum likelihood (REML) for the following model structures:

$$\text{fixed : genotype}^* \text{ location}^* \text{ depth}^* \text{ age} \quad (3)$$

$$\text{random : block/plot/location/depth/age} \quad (4)$$

$$\text{variance : block} \times \text{plot} \times \text{location} \times \text{depth} \times \text{age} \quad (5)$$

where 'genotype', 'depth' and 'age' were self-explanatory, 'location' allocated data to either *plant* or *gap*, and '\*', '/' and ' $\times$ ' represent all cross-products, nesting and interactions of the factors, respectively. We introduced the variance structure above for all analyses as there was no independence of samples at different depths from each treatment in the strictest

sense because a single soil core was taken and subdivided into depth samples. We may expect correlations between pairs of depths for any property to vary rather than be constant, depending on the distance between them. Therefore, we introduced an autoregressive variance structure into our REML models to incorporate this feature. For the SOC and TN stocks and their rate of change, neither data transformation nor the variance structure were required, 'period' (i.e. 0–4 and 4–6 years) replaced 'age' for the rates of change, and 'location' or 'depth' were removed when the data were composited into larger-scale spatial and full 1 m depth scales. We present the statistical analysis of all transformed data (where required) in a summary table but present the original data elsewhere for ease of displaying measured quantities. The Wald statistic produced during REML analysis assesses the contributions of individual terms in the fixed model and corresponds to the treatment sum of squares divided by the stratum mean square.

## Results

### Root Density

The full genotype  $\times$  location  $\times$  depth  $\times$  age interaction was significant ( $P=0.014$ ) for root density (Table 2; Fig. 1). For Sinensis, root density was significantly greater ( $P<0.05$ ) for *plant* ( $6.6 \pm 1.5$  after 4 years and  $15.5 \pm 3.1 \text{ kg m}^{-3}$  after 6 years; mean  $\pm$  standard error) compared to *gap* ( $1.4 \pm 0.4$  after 4 years and  $2.2 \pm 0.4 \text{ kg m}^{-3}$  after 6 years) at 0–0.1 m depths. The same was also true for Sinensis at 0.1–0.2 m depth ( $2.0 \pm 0.2$  after 4 years and  $7.2 \pm 2.5 \text{ kg m}^{-3}$  after 6 years for *plant*, and  $0.4 \pm 0.1$  after 4 years and  $1.2 \pm 0.2 \text{ kg m}^{-3}$  after 6 years for *gap*) ( $P<0.05$ ). Root density was significantly greater under Giganteus at 0.1–0.2 m depth after 6 years for *plant* ( $4.4 \pm 1.0 \text{ kg m}^{-3}$ ) than *gap* ( $0.8 \pm 0.2 \text{ kg m}^{-3}$ ) ( $P<0.05$ ). Significant increases ( $P<0.05$ ) in root density between 4 and 6 years were found for Sinensis *gap* at 0.2–0.3 ( $0.2 \pm 0.1$  to  $0.9 \pm 0.2 \text{ kg m}^{-3}$ ) and 0.3–0.5 m depths ( $0.1 \pm 0.1$  to  $0.4 \pm 0.1 \text{ kg m}^{-3}$ ), and for Tora *plant* at 0.2–0.3 m depth ( $0.2 \pm 0.1$  to  $1.1 \pm 0.6 \text{ kg m}^{-3}$ ). Significant genotype differences were apparent, but these were mainly restricted to upper layers, where density was often greater for *Miscanthus* (particularly Sinensis) than willow varieties. Root density decreased with depth for all genotypes.

### Root Diameter and RLD

Mean root diameter was significantly greater ( $P<0.05$ ) for Giganteus ( $0.55 \pm 0.01 \text{ mm}$ ) and, at most depths, Sinensis ( $0.47 \pm 0.02 \text{ mm}$ ) compared to both willow varieties ( $0.35 \pm 0.02 \text{ mm}$ ) (Table 2; Fig. 2). Significant differences in root diameter between *plant* and *gap* were restricted to the 0–0.1 m depth for Sinensis and Tora. For Sinensis, roots in *plant* were

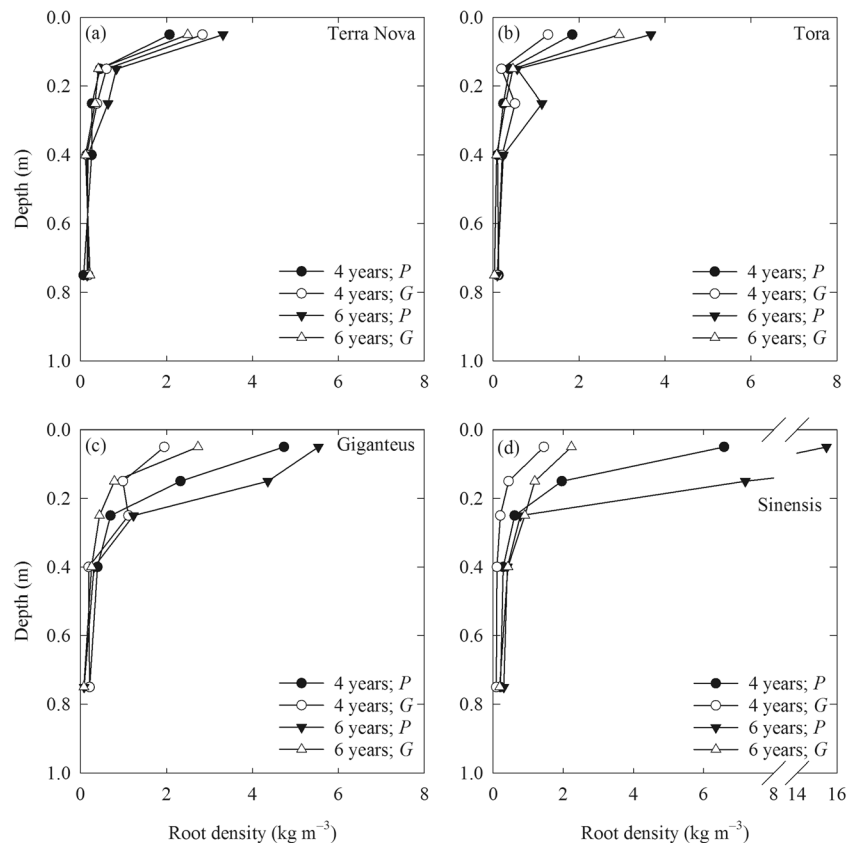
**Table 2** The residual maximum likelihood (REML) table for the root and soil variates with the REML structures outlined in Eqs. 3–5 showing just the genotype (G), location (L), depth (D) and age (A) factors or the lowest-level fixed term interactions (×) that were significant ( $P < 0.05$ )

Variate	Unit	Fixed term	Wald	n.df	F	d.df	P value	SED
Root density	$\log_{10}(\text{kg m}^{-3})$	$G \times L \times D \times A$	25.28	12	2.11	120	0.014	0.317
Mean root diameter	$\log_{10}(\text{mm})$	$L \times A$	4.37	1	4.37	120	0.041	0.018
		$G \times L \times D$	27.56	12	2.29	96	0.014	0.051
		$G \times D \times A$	31.07	12	2.57	120	0.005	0.047
Root length density	$\log_{10}(\text{cm cm}^{-3})$	$L$	6.97	1	6.97	12	0.008	0.048
		$G \times D$	27.20	12	2.27	96	0.007	0.128
		$G \times A$	10.35	3	3.45	120	0.016	0.120
SOC density	$\log_{10}(\text{kg m}^{-3})$	$D \times A$	13.74	4	3.44	120	0.008	0.023
<i>Miscanthus</i> -derived SOC density	$\log_{10}(\text{kg m}^{-3})$	$G \times L \times D$	11.88	4	2.96	48	0.024	0.173
		$G \times D \times A$	12.89	4	3.21	60	0.016	0.173
TN density	$\log_{10}(\text{kg m}^{-3})$	$G$	8.80	3	2.93	9	0.032	0.025
		$L \times D \times A$	11.13	4	2.78	120	0.025	0.037
SOC/TN ratio	$\log_{10}(\text{ratio})$	$L \times D$	19.61	4	4.90	96	< 0.001	0.016
		$G \times A$	14.72	3	4.91	120	0.003	0.017
		$D \times A$	20.63	4	5.16	120	< 0.001	0.016

The table gives the Wald statistic, the degrees of freedom (df) associated with the numerator (n) and denominator (d), the variance ratio statistic ( $F$ ), the probability level associated with  $F$  ( $P$ ) and the standard error of differences (SED). Note that all variates were transformed by  $\log_{10}$  firstly to normalise the distribution of residuals and hence the SED is also expressed in the transformed unit

coarser (0.51–0.81 mm) than in *gap* (0.34–0.43 mm), whereas (0.32–0.90 mm) ( $P < 0.05$ ). Mean root diameter significantly increased between 4 and 6 years for all genotypes at all depths for Tora roots in *plant* were finer (0.26–0.48 mm) than in *gap*

**Fig. 1** Mean root density ( $n = 4$ ) under willow (a Terra Nova and b Tora) and *Miscanthus* (c Giganteus and d Sinensis) genotypes, located either directly under the plant ( $P$ ) or in the gap between adjacent plants ( $G$ ), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the genotype  $\times$  location  $\times$  depth  $\times$  age interaction was significant ( $P = 0.014$ ) (see Table 2)



( $P < 0.05$ ) with few exceptions, and was generally greatest in the 0–0.1 m depth. The RLD was significantly greater ( $P < 0.05$ ) for *plant* ( $3.9 \pm 0.3 \text{ cm cm}^{-3}$ ) compared to *gap* ( $3.1 \pm 0.3 \text{ cm cm}^{-3}$ ) averaged over all other factors and decreased significantly with depth (Table 2; Fig. 3). *Giganteus* had the smallest ( $0.2\text{--}9.9 \text{ cm cm}^{-3}$ ) and *Sinensis* the greatest RLD ( $0.4\text{--}11.4 \text{ cm cm}^{-3}$ ) at most depths. Only for *Sinensis* was there a significant increase ( $P < 0.05$ ) in RLD between 4 and 6 years ( $2.7 \pm 0.4$  to  $4.9 \pm 0.6 \text{ cm cm}^{-3}$ ).

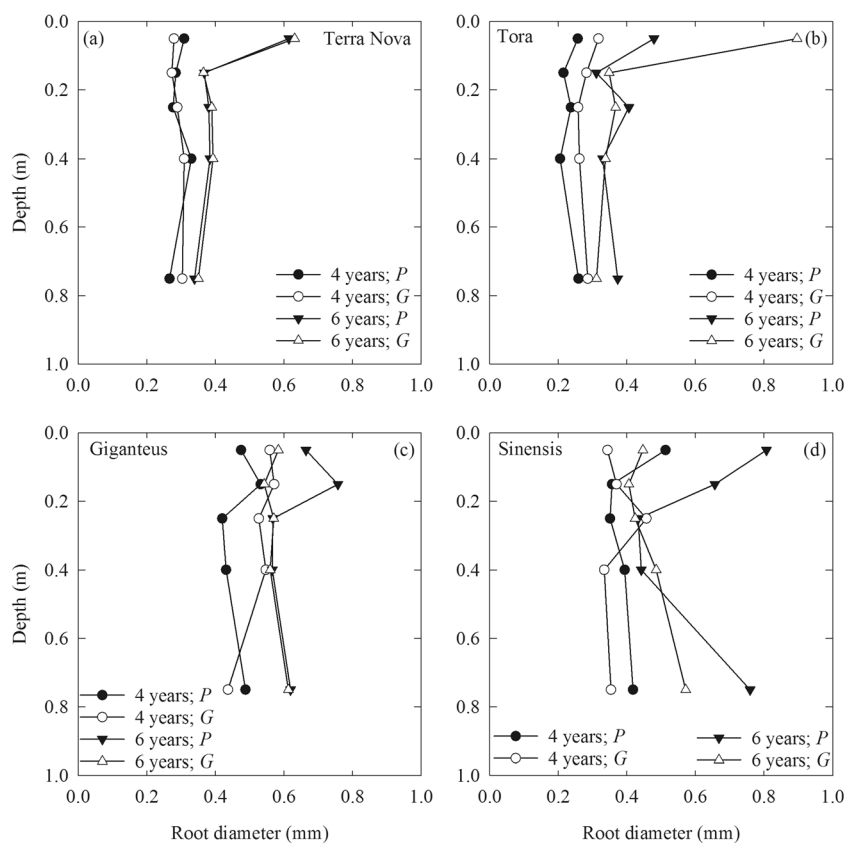
## SOC and TN Density

The SOC density increased significantly ( $P < 0.05$ ) between 4 and 6 years at all depths below 0.2 m (from  $3.6 \pm 0.1$  to  $12.2 \pm 0.7 \text{ kg m}^{-3}$  after 4 years to  $4.6 \pm 0.1$  to  $14.3 \pm 0.6 \text{ kg m}^{-3}$  after 6 years, averaged over all other factors) (Table 2; Fig. 4). Genotype and location effects were not significant. The SOC density after 4 and 6 years was similar to the initial baseline (0 years) in the upper 0.5 m, though it was much greater under *Sinensis* and *Tora* after 6 years in the upper 0.2 m, but less than initial density below 0.5 m (Fig. 4). *Miscanthus*-derived SOC density was significantly greater ( $P < 0.05$ ) under *Sinensis plant* ( $7.4 \pm 2.1$  to  $11.6 \pm 1.8 \text{ kg m}^{-3}$ ) than *Giganteus plant* in the 0–0.1 m depth ( $2.9 \pm 0.4$  to  $4.8 \pm 0.5 \text{ kg m}^{-3}$ ), and significantly greater under *Giganteus gap* ( $0.1 \pm 0.0$  to  $0.5 \pm 0.1 \text{ kg m}^{-3}$ ) than *Sinensis gap* at 0.5–1.0 m depth ( $0.0 \pm 0.0$  to  $0.5 \pm$

$0.0 \text{ kg m}^{-3}$ ) (Table 2; Fig. 5). *Miscanthus*-derived SOC density was also greater for *plant* than *gap* for *Sinensis* at most depths ( $P < 0.05$ ), whereas differences between *plant* and *gap* were not significant for *Giganteus*. Averaged over both locations, *Miscanthus*-derived SOC density increased significantly ( $P < 0.05$ ) between 4 and 6 years at all depths under both genotypes (by  $0.4\text{--}3.2 \text{ kg m}^{-3}$ ), with few exceptions.

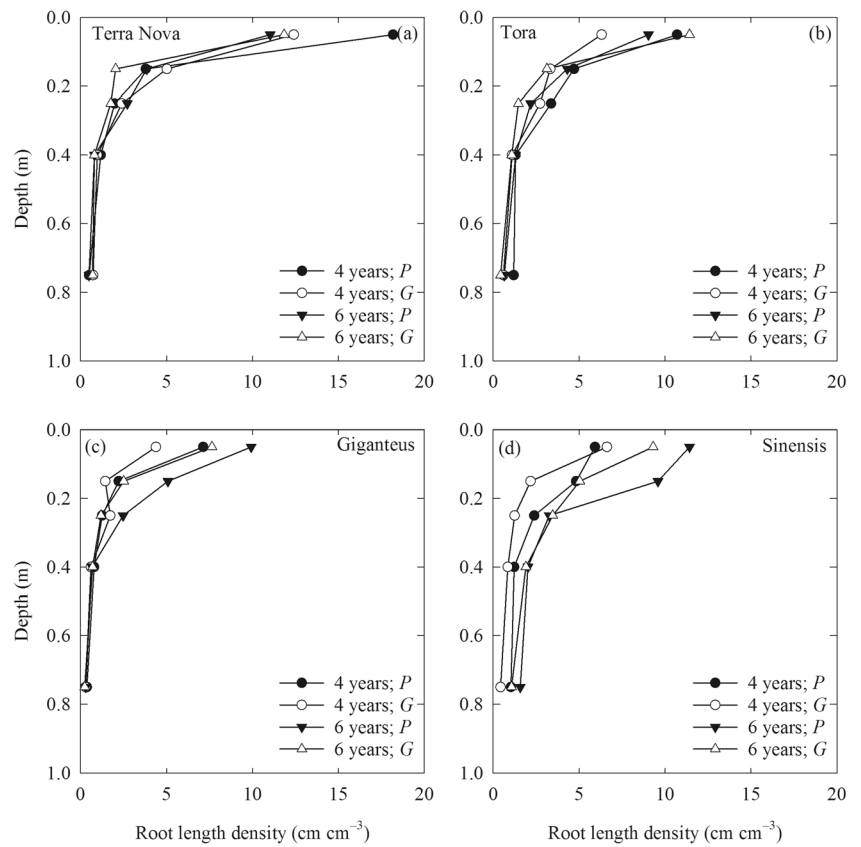
The TN density after 4 years was significantly greater ( $P < 0.05$ ) for *gap* ( $2.2 \pm 0.1 \text{ kg m}^{-3}$ ) than *plant* ( $1.7 \pm 0.1 \text{ kg m}^{-3}$ ) at 0–0.2 m depths, but significantly less ( $P < 0.05$ ) for *gap* ( $0.6 \pm 0.0 \text{ kg m}^{-3}$ ) than *plant* ( $0.8 \pm 0.1 \text{ kg m}^{-3}$ ) at 0.5–1.0 m depth (Table 2; Fig. 6). Averaged over all other factors, there was a significantly greater ( $P < 0.05$ ) TN density associated with *Sinensis* and *Terra Nova* ( $1.4 \pm 0.1 \text{ kg m}^{-3}$ ) compared to *Giganteus* ( $1.2 \pm 0.1 \text{ kg m}^{-3}$ ). Compared to the initial baseline, only for *Terra Nova* and *Sinensis* was there a greater TN density recorded after 4 years at certain depths. There were no significant changes between 4 and 6 years. The SOC/TN ratio increased significantly ( $P < 0.05$ ) from 4 to 6 years under all genotypes (by 1.2–1.6) except *Giganteus*, and at all depths (by 0.7–1.6) except 0–0.1 m (Fig. 7). Only at 0–0.2 m depths was there a significantly greater SOC/TN ratio for *plant* compared to *gap* (by 0.9–2.1). The SOC and TN densities and their ratio decreased significantly with depth ( $P < 0.05$ ). The SOC/TN ratio after 4 and 6 years under bioenergy crops was greater than that

**Fig. 2** Mean root diameter ( $n = 4$ ) under willow (a *Terra Nova* and b *Tora*) and *Miscanthus* (c *Giganteus* and d *Sinensis*) genotypes, located either directly under the plant (*P*) or in the gap between adjacent plants (*G*), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the location  $\times$  age ( $P = 0.041$ ), genotype  $\times$  location  $\times$  depth ( $P = 0.014$ ) and genotype  $\times$  depth  $\times$  age ( $P = 0.005$ ) interactions were significant (see Table 2)





**Fig. 3** Mean root length density ( $n = 4$ ) under willow (**a** Terra Nova and **b** Tora) and *Miscanthus* (**c** Giganteus and **d** Sinensis) genotypes, located either directly under the plant ( $P$ ) or in the gap between adjacent plants ( $G$ ), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the location factor ( $P = 0.008$ ) and genotype  $\times$  depth ( $P = 0.007$ ) and genotype  $\times$  age ( $P = 0.016$ ) interactions were significant (see Table 2)



in the baseline in the upper soil layers. The original gravimetric SOC and TN concentrations ( $\text{g kg}^{-1}$ ) are given elsewhere (Supplementary Material).

### SOC and TN Stock

Stocks of SOC increased significantly ( $P < 0.05$ ) in the 0.3–1.0 m depth between 4 and 6 years from  $29 \pm 2$  to  $38 \pm 2 \text{ Mg ha}^{-1}$ , averaged over all crops, whereas TN stocks were unaffected by any factor (Table 3). Stocks of SOC in the full 1 m profile changed from  $79 \pm 4$  to  $81 \pm 5 \text{ Mg ha}^{-1}$  after 4 years to  $87 \pm 5$  to  $96 \pm 4 \text{ Mg ha}^{-1}$  after 6 years, and TN stocks in the full 1 m profile changed from  $9.2 \pm 0.9$  to  $11.0 \pm 0.5 \text{ Mg ha}^{-1}$  after 4 years to  $9.4 \pm 0.5$  to  $10.8 \pm 0.4 \text{ Mg ha}^{-1}$  after 6 years. Genotype-specific differences were not significant however. *Miscanthus*-derived SOC increased significantly ( $P < 0.05$ ) from  $3.4 \pm 0.5$  to  $5.2 \pm 0.6 \text{ Mg ha}^{-1}$  after 4 years to  $11.4 \pm 0.8$  to  $14.2 \pm 1.7 \text{ Mg ha}^{-1}$  after 6 years. For both SOC and TN, stocks in the 0–0.3 m depth were greater and stocks in the 0.3–1.0 m depth were lower after 4 and 6 years compared to those estimated for the baseline. Between years 4 and 6, SOC increased to 1 m depth at  $6.4 \pm 1.9$  for Terra Nova,  $7.4 \pm 2.5$  for Tora,  $3.1 \pm 2.1$  for Giganteus and  $8.8 \pm 3.4 \text{ Mg ha}^{-1} \text{ year}^{-1}$  for Sinensis, being greater significantly ( $P < 0.05$ ) for the 0.3–1.0 m depth, averaged over genotypes. Stocks of TN increased slightly by  $0.1 \pm 0.3 \text{ Mg ha}^{-1} \text{ year}^{-1}$  except for Terra Nova

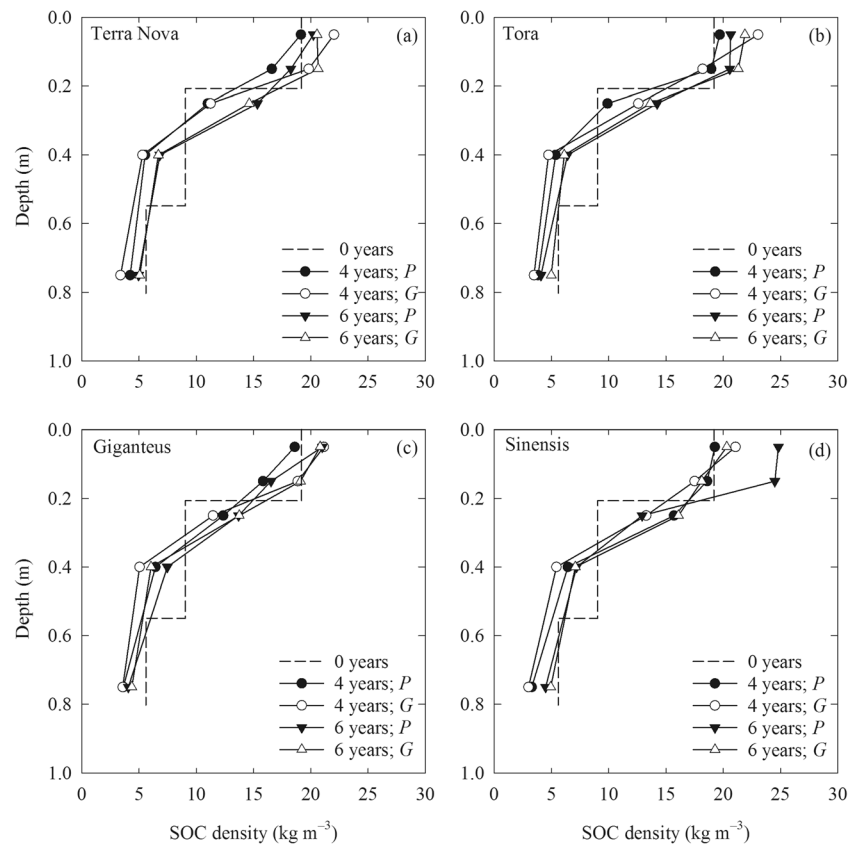
where it declined by  $-0.15 \pm 0.2 \text{ Mg ha}^{-1} \text{ year}^{-1}$  over the same period. Generally, rates of change were greater for the 4- to 6-year period than the 0- to 4-year period. Location-based SOC and TN stocks and rates of change are given elsewhere (Supplementary Material).

## Discussion

### Bioenergy Crop Root Characteristics

Root mass was greater under *Miscanthus* compared to willow, although such differences were restricted to the upper 0.3 m. Root mass below 0.3 m was very small under all genotypes due in part to the high clay content of the subsoil ( $> 26\%$ ) where the ability of roots to penetrate was likely restricted to existing structures such as shrinkage cracks [32]. Only for *Miscanthus* was a spatial pattern between root mass and location, with respect to canopy structure, identified whereby distinct *plant* and *gap* zones down to 0.2 m depth developed, particularly under Sinensis. Sinensis is a tuft-forming genotype, especially during establishment, and its areal coverage in the *gap* does not increase as much as Giganteus with age [7, 23]. Only for Tora in the upper 0.1 m was there a suggestion of an effect of location with respect to willow root mass. We were not able to sample from directly over the centre of the willow plants; therefore, our

**Fig. 4** Mean soil organic C (SOC) density ( $n = 4$ ) under willow (**a** Terra Nova and **b** Tora) and *Miscanthus* (**c** Giganteus and **d** Sinensis) genotypes, located either directly under the plant (*P*) or in the gap between adjacent plants (*G*), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the depth  $\times$  age interaction was significant ( $P = 0.008$ ), but neither genotype nor location factors were (see Table 2). The baseline data (0 years) for three depth intervals (adjusted for equivalent soil mass) is shown for comparison

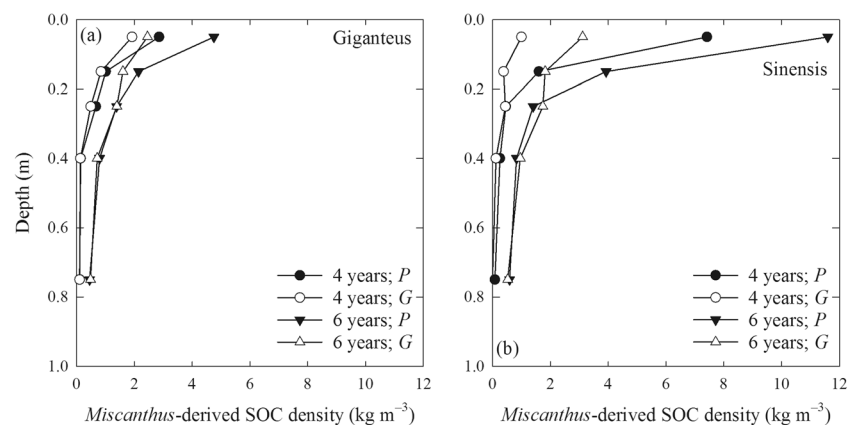


plant sample under willow differs slightly from that under *Miscanthus* which may partly mask the lack of effect of location, although RLD was greater for *plant* compared to *gap* for both bioenergy crop species. Observed growth of other species (weeds) between willow plants may have provided a confounding source of roots that could not be distinguished using bulk measurements. Nevertheless, the results indicate that willow roots spread laterally as the stand matures [33].

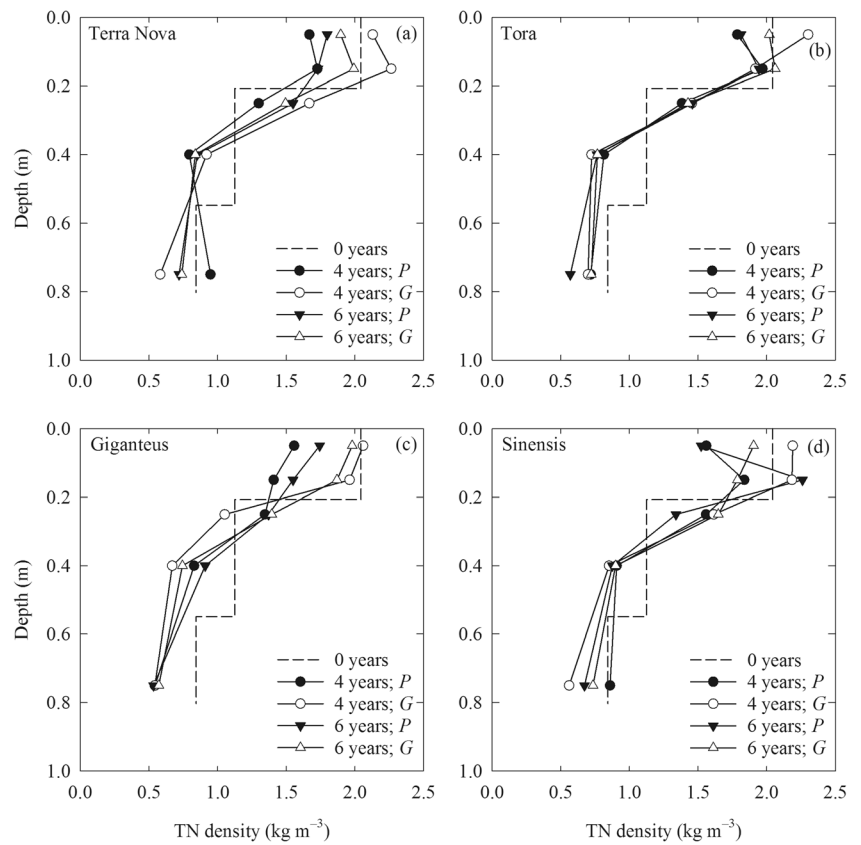
Root mass and RLD increased substantially over the 2-year period in the upper 0.2 m between measurements under Giganteus and, especially, Sinensis. Continual growth of roots under *Miscanthus* was expected as the stand was still maturing

during this period [34], with each new shoot developing its own root system. Root biomass may also increase for *Miscanthus* during periods of water stress [1]. Although both 2014 and 2015 recorded greater-than-average (1981–2010) annual temperature and rainfall (+1.5 °C and +191 mm in 2014; +0.7 °C and +48 mm in 2015), in March and April prior to sampling in 2015, rainfall had been up to 50% lower than average [29]. The root system of willows, particularly under the 2-year SRC regime, may have developed to the full extent prior to the first measurement after 4 years such that subsequent root growth was balanced by turnover, as observed by others [35]. Total above-ground yield for both willow varieties

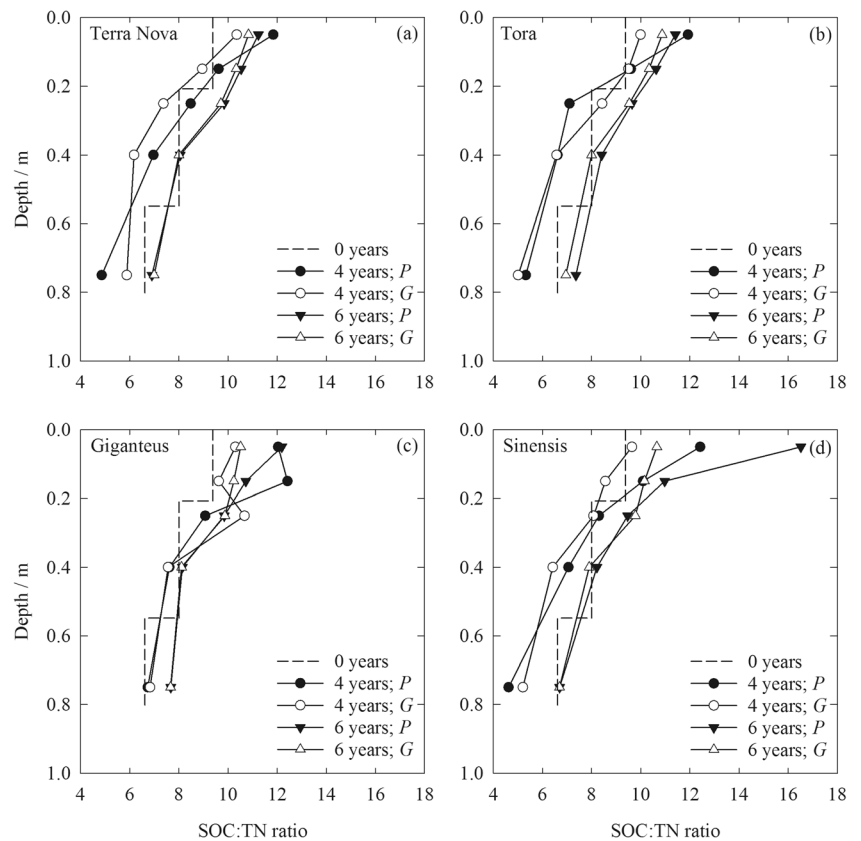
**Fig. 5** Mean *Miscanthus*-derived soil organic C (SOC) density ( $n = 4$ ) under *Miscanthus* (**a** Giganteus and **b** Sinensis) genotypes, located either directly under the plant (*P*) or in the gap between adjacent plants (*G*), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the genotype  $\times$  location  $\times$  depth ( $P = 0.024$ ) and genotype  $\times$  depth  $\times$  age ( $P = 0.016$ ) interactions were significant (see Table 2)



**Fig. 6** Mean soil total N (TN) density ( $n = 4$ ) under willow (**a** Terra Nova and **b** Tora) and *Miscanthus* (**c** Giganteus and **d** Sinensis) genotypes, located either directly under the plant ( $P$ ) or in the gap between adjacent plants ( $G$ ), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the genotype factor ( $P = 0.032$ ) and location  $\times$  depth  $\times$  age interaction ( $P = 0.025$ ) were significant (see Table 2). The baseline data (0 years) for three depth intervals (adjusted for equivalent soil mass) is shown for comparison



**Fig. 7** Mean soil organic C/total N ratio (SOC:TN) ( $n = 4$ ) under willow (**a** Terra Nova and **b** Tora) and *Miscanthus* (**c** Giganteus and **d** Sinensis) genotypes, located either directly under the plant ( $P$ ) or in the gap between adjacent plants ( $G$ ), when the stand age was 4 and 6 years. With the data transformed ( $\log_{10}$ ), the location  $\times$  depth ( $P < 0.001$ ), genotype  $\times$  age ( $P = 0.003$ ) and depth  $\times$  age ( $P < 0.001$ ) interactions were significant (see Table 2). The baseline data (0 years) for three depth intervals (adjusted for equivalent soil mass) is shown for comparison





decreased from about 25 Mg ha<sup>-1</sup> in 2012–2013 to 22 Mg ha<sup>-1</sup> in the 2014–2015 SRC growth cycle, whereas biennial production increased under *Giganteus* (26 to 34 Mg ha<sup>-1</sup>) and *Sinensis* (18 to 20 Mg ha<sup>-1</sup>) over the same period. This would appear to support the differences in root biomass described above. Willow roots were finer than *Miscanthus* roots which may increase their turnover rate [15], although mean root diameter under all genotypes increased in size over time, and RLD did not vary much over the measurement period. Despite measurable differences in some above-ground traits of willows [5], these did not appear to be manifest in differences in the root traits we measured in this study.

Our estimates of root biomass are comparable with other studies. Ferchaud et al. [22] reported root biomass of 4.1 Mg ha<sup>-1</sup>, assuming a C content of 43% [15], under a 5-year stand of *Giganteus* in northern France under similar climatic conditions, similar to our measurement (2.9–7.1 Mg ha<sup>-1</sup>) when root density is expressed as a stock. The rhizome biomass of 18.1 Mg ha<sup>-1</sup> given by Ferchaud et al. [22] is within the wide range of that calculated for the 4-year-old *Giganteus* herein (6.4 in *gap* and 109.3 Mg ha<sup>-1</sup> in *plant*). The rhizome itself may accumulate more than 1 Mg C ha<sup>-1</sup> year<sup>-1</sup> under established *Miscanthus* stands, providing a larger store of C than the root system (<1 Mg C ha<sup>-1</sup> year<sup>-1</sup>), according to Agostini et al. [15].

### SOC and TN under Bioenergy Crops

We found that SOC increased over time under all genotypes below, though not above, 0.2 m depth. Differences in topsoil SOC gravimetric concentration (g kg<sup>-1</sup>; [Supplementary Material](#)) were not manifest in differences in volumetric density due to bulk density (which was significantly lower ( $P < 0.05$ ) under *plant* at 0–0.1 m, averaged over all genotypes), as observed previously [23]. Concentrations of SOC matched patterns in root concentration and density, where regressions explained 0.58 (concentration) and 0.55 (density) of the proportion of the variance ([Supplementary Material](#)). This was most apparent for *Miscanthus* where there was strong evidence linking *Miscanthus*-derived SOC to root mass, with regressions explaining up to 0.79 proportion of the variance ([Supplementary Material](#)), though not obviously RLD. This supports the hypothesis that roots were the main source of SOC under bioenergy crops [8], assumed to be facilitated by the reallocation of photosynthate to the root and rhizome at senescence. Similar mechanisms occur with respect to the stools of willow [1, 33].

Whilst there may be some inputs to the soil from leaf litter under willow, this may be recycled back into the plant rapidly as established stands effectively provide their own nutrient needs through internal cycling [1]. Rubino et al. [36] quantified significant incorporation of <sup>13</sup>C-labelled poplar litter into the topsoil horizon in Italy. Significant input from *Miscanthus* leaves is unlikely, however [18], despite a potential input of up

to 7 Mg C ha<sup>-1</sup> year<sup>-1</sup> [37–39]. Even for late-harvest *Miscanthus*, leaf fall remains largely undecomposed [22], partly because of its reduced quality arising from the translocation of N from senescing leaves to the rhizome at the end of the growing season [40]. This supports below-ground biomass as the primary source of SOC.

Above-ground yields over the 2012–2016 period were greatest for *Giganteus* (15 Mg ha<sup>-1</sup> year<sup>-1</sup>) and least for *Sinensis* (9 Mg ha<sup>-1</sup> year<sup>-1</sup>), with the willow varieties being intermediate (12 Mg ha<sup>-1</sup> year<sup>-1</sup>), yet this was not reflected in root biomass or SOC. As TN did not change significantly over time, the SOC/TN ratio increased, particularly in the top 0.2 m under *Sinensis*. This may reflect the high C/N ratio of the source material: the C/N ratio of *Miscanthus* roots can exceed 40 (data not presented) suggesting that microbial N mining may control the decomposition of organic C, thereby increasing potential C sequestration rates, and the maintenance of poor TN contents in this N-limited system [41].

Inputs of C to the soil may be important when willow is coppiced as increased root turnover may follow harvesting of above-ground biomass [2]. Comparable SOC sequestration rates of 3.4 Mg ha<sup>-1</sup> year<sup>-1</sup> in the upper 1 m under SRC willow in the UK [42] and up to 6.7–10.2 Mg ha<sup>-1</sup> year<sup>-1</sup> in the upper 0.6 m of soil under SRC willow and poplar in Belgium were recorded recently [43]. Others have reported comparable SOC sequestration rates below willow stands of 1.0 Mg ha<sup>-1</sup> year<sup>-1</sup> in the upper 0.1 m over a 6-year period in Italy [44] and 1.5–1.7 Mg ha<sup>-1</sup> year<sup>-1</sup> in the upper 0.9 m over an 11-year period in Germany [45]. Lesser accumulations of 0.2–0.3 Mg ha<sup>-1</sup> year<sup>-1</sup> were reported at 0–0.25 m depth under a 12-year willow stand in Germany [46], whilst others have reported losses of SOC under willow [47], particularly on former grassland [18, 48]. On average, a lower mean annual SOC sequestration rate under willow of 0.6 Mg ha<sup>-1</sup> year<sup>-1</sup> has been reported [15].

Determining the stable <sup>13</sup>C isotope signature of SOC from *Miscanthus* stands confirms that fresh *Miscanthus*-derived C was progressively added to the soil since planting in 2009. *Giganteus* had a more extensive effect initially on increasing SOC stocks, but *Sinensis* became more effective at increasing SOC stocks. Equivalent *Miscanthus*-derived SOC accumulation rates in the 0–0.3 m depth during the first 4 years were 1.1 for *Giganteus* and 0.8 Mg ha<sup>-1</sup> year<sup>-1</sup> for *Sinensis*. In an older stand of *Miscanthus* at the same site, Richter et al. [23] reported accumulations of 1.2 under *Giganteus* and 0.7 Mg ha<sup>-1</sup> year<sup>-1</sup> under *Sinensis* over 14 years. Our rates are comparable to those measured by others for topsoil under *Giganteus* in France (0.4–0.7 Mg ha<sup>-1</sup> year<sup>-1</sup>; [22]), Ireland (0.6 Mg ha<sup>-1</sup> year<sup>-1</sup>; [12, 34]), Germany (0.7 Mg ha<sup>-1</sup> year<sup>-1</sup>; [45]), the USA (1.1 Mg ha<sup>-1</sup> year<sup>-1</sup>; [49]), Italy (1.2 Mg ha<sup>-1</sup> year<sup>-1</sup>; [50]), the UK (1.4 Mg ha<sup>-1</sup> year<sup>-1</sup>; [20]) and various sites in Europe (0.4–1.0 Mg ha<sup>-1</sup> year<sup>-1</sup>; [17]), and similar to recent global estimates of sequestration under *Miscanthus* of around 1.2 Mg ha<sup>-1</sup> year<sup>-1</sup> [15, 51].



Data on SOC changes below 0.3 m are limited, but our estimated *Miscanthus*-derived SOC rates of up to  $0.2 \text{ Mg ha}^{-1} \text{ year}^{-1}$  over the initial 4 years for both genotypes are similar to those reported in a global review of *Giganteus* planted following arable cropping ( $0.1 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ; [51]) and studies in Italy ( $0.5 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ; [44]) and the UK ( $0.1 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ; [23]). For the following 2 years, we report considerably greater *Miscanthus*-derived SOC accumulation rates of 1.5 (*Giganteus*) and  $3.3 \text{ Mg ha}^{-1} \text{ year}^{-1}$  (*Sinensis*) in the 0–0.3 m depth and 1.6 (*Giganteus*) and  $2.1 \text{ Mg ha}^{-1} \text{ year}^{-1}$  (*Sinensis*) in the 0.3–1.0 m depth. These rates are still comparable, however, to others reported in the literature. Rates of up to 6.8 for a 4–5-year-old stand and  $8.8 \text{ Mg ha}^{-1} \text{ year}^{-1}$  for an 8–9-year-old stand were recorded in the upper 0.25 m under *Giganteus* in Germany [52], and rates of up to  $4 \text{ Mg ha}^{-1} \text{ year}^{-1}$  in the topsoil were reported for grass bioenergy crops in general [8].

Increases in SOC stock observed under *Miscanthus* were not wholly explained by *Miscanthus*-derived inputs, suggesting an input of C to the system from another (non-C<sub>4</sub>) source. On occasions, we observed other plants to be present on the plot, despite weed control, though we could not quantify the areal extent. Other factors include trends in atmospheric CO<sub>2</sub> [53, 54], and variations in plant input signatures relating to physiological stress [55, 56], which all affect the  $\delta^{13}\text{C}$  determinations used to estimate *Miscanthus*-derived inputs. The former would be unlikely to have been significant given the short period between measurements, but the latter may have been important. In the 0–0.3 m depth of *gap*, however, we did estimate a greater accumulation rate of *Miscanthus*-derived SOC than total SOC, which would indicate turnover of existing C<sub>3</sub> plant-derived SOC. Preferential processing of inherent SOC leading to persistence of *Miscanthus*-derived SOC and an overall balance in SOC have been observed previously [22, 57], and have led to a caveat that effective sequestration may be marginal [18, 23].

Temporal changes in soil TN were largely insignificant, but interesting differences were observed between genotypes. There were marginal increases in TN in topsoil for *plant* (up to  $0.13 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) and marginal decreases in *gap* (down to  $-0.31 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ), but the opposite in the 0.3–1.0 m depth (down to  $-0.38$  for *plant* and up to  $0.41 \text{ Mg TN ha}^{-1} \text{ year}^{-1}$  for *gap*). We suggest that processes such as uptake of N by lateral roots in the *gap*, loss through leaching down the profile into the subsoil (where not directly mediated by the plant) and loss through denitrification (waterlogging has been observed at the field site in very wet periods) may have been responsible. Increased TN for *plant* probably derived from the same residues associated with SOC increases, albeit at much lower rates due to the relatively large C/N ratio of the plant inputs.

We estimate that by year 6, SOC was sequestered at a rate of 3 to  $9 \text{ Mg SOC ha}^{-1} \text{ year}^{-1}$  under all crops in the upper 1 m,

following an apparent loss of SOC in the first 4 years specific to the 0.3–1.0 m depth. That SOC may be lost in the establishment phase of bioenergy crop stands is normal, but we must be cautious with the baseline data. Stocks in 2009 prior to establishment were calculated from separate samples collected on separate occasions for SOC concentration and bulk density. The bulk density values in 2009 ( $1.47\text{--}1.61 \text{ Mg m}^{-3}$ ) were greater than average bulk densities in 2013 and 2015 ( $1.22\text{--}1.41 \text{ Mg m}^{-3}$ ). It was likely that measured bulk density was greater in 2009 in part because samples were collected in smaller cores presumably with less chance of including > 2 mm stones that are otherwise accounted for and removed. Additionally, the depth intervals in 2009 did not correspond to those in 2013 and 2015 which required adjustment pro rata. Nevertheless, we report an increase in SOC accumulation rates under all bioenergy crops with time. After 6 years, approximately 14% of SOC ( $11$  to  $14 \text{ Mg ha}^{-1}$ ) had derived from *Miscanthus*. Soil TN was a different matter, and we estimated losses of up to  $-0.15 \text{ Mg TN ha}^{-1} \text{ year}^{-1}$  under Terra Nova but marginal increases of up to  $1.0 \text{ Mg TN ha}^{-1} \text{ year}^{-1}$  under the other crops. We may presume that high N requirements of willows [58] were met in part by soil TN reserves in addition to fertiliser.

Previous land use is important for a full evaluation of sequestration potential [8, 16]. In their review of global datasets, Qin et al. [51] reported greater accumulation rates of SOC where willow and *Miscanthus* were planted on former arable land, compared to grassland where some net losses were recorded. This has been supported by paired-site studies in the UK [18]. Former grassland soils have been observed to recover such lost SOC in the medium term however [12, 42]. Undoubtedly, some SOC was lost following initial cultivation of the field site, but after the first few SRC cycles of willow, or the first few harvests of *Miscanthus*, we may expect the SOC stock to increase such that the initial losses are regained, particularly as the site was previously under arable cropping. We therefore expect C to have been sequestered in the soils under bioenergy crops, compared to the previous arable land use, over the lifetime of the plantation [8].

We observed soil SOC accumulation rates exceeding by far a proposed C-neutrality threshold of  $0.25 \text{ Mg ha}^{-1} \text{ year}^{-1}$  [15, 24]. Indeed, if our rates of  $3.09\text{--}8.84 \text{ Mg C ha}^{-1} \text{ year}^{-1}$  are converted firstly to CO<sub>2</sub> equivalents ( $11.3\text{--}32.4 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ), we may then use the 100-year global warming potential factor of 298 to calculate the equivalent N<sub>2</sub>O emission threshold that should not be exceeded to maintain overall greenhouse gas mitigation. This estimate of  $38\text{--}109 \text{ kg N}_2\text{O ha}^{-1} \text{ year}^{-1}$  is far greater than current estimates of emissions under both arable ( $0.9\text{--}11.0 \text{ kg N}_2\text{O ha}^{-1} \text{ year}^{-1}$ ) and grassland agriculture ( $1.6\text{--}22.0 \text{ kg N}_2\text{O ha}^{-1} \text{ year}^{-1}$ ) in the UK [59]. Sequestration in the subsoil may be especially important as such SOC may become protected against further processing [60].

Although our SOC accumulation rates are greater than others (e.g. [17, 48]), they are not unprecedentedly so (e.g. [43, 52]), and the rates of change relative to initial SOC stocks are comparable elsewhere. Poehlau et al. [61] calculated accumulations of SOC of 10 to 50% within 10 years from arable to grass and arable to woodland land use conversions, which covers the kinds of proportional increases we found here (up to 12% increase in the 0–0.3 m depth and up to 43% increase in the 0.3–1.0 m depth from 4 to 6 years). The subsoil may also be affected by land use change [61, 62] and accumulation rates may be greater where the subsoil SOC stock was low initially [17]. Berhongaray et al. [43] also calculated greater sequestration rates under SRC willow and poplar at 0.3–0.6 m depth compared to 0–0.3 m depth. Not all studies sample to the same depths as this present study and hence may miss SOC changes at depth. We ascribe our greater accumulation rates under both bioenergy crops to the younger age of the stand and the low initial SOC status of the soil which had been under long-term arable management prior.

## Conclusions

Genotypes of willow and *Miscanthus* sequestered SOC in an underlying temperate agricultural silty clay loam soil with the root system being the most likely source. Soil TN was little-affected and hence addition of fresh residues to the soil increased the SOC/TN under all genotypes. This may lead to persistence of the new residues, although isotopic evidence for turnover of the inherent SOC in preference to fresh *Miscanthus*-derived SOC was limited, perhaps due to inputs from undergrowth species. Both bioenergy crops offer the double benefit of biomass production and C sequestration when planted in arable soil initially low in SOC. Future studies will continue to monitor changes in SOC and TN to assess the dynamics reported here and will exploit isotopic and biochemical approaches to focus on turnover rates of above- and below-ground plant constituents over the full life cycle of bioenergy crop stands.

**Acknowledgements** We thank the following colleagues at Rothamsted Research for their help with this work: A Karp, PJ Murray (planning and discussion); TJP Barraclough, M Castle, I Durenkamp, P Fruen (sampling); M Masna, DJW Steele (sample processing and root analysis); M Brookman, A Duffy, M Redmile-Gordon, R Skilton and X Zhou (soil analysis); farm staff (maintaining the field experiments). We also thank two anonymous reviewers and the editors for their constructive comments in improving the paper.

**Funding Information** This work formed part of the ‘Maximising carbon retention in soils’ project (BBS/E/C/00005214) funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC). The field experiment was established under the Bioenergy from *Miscanthus* And *Salix* Species (BioMASS) project (BB/G016216/1) of the BBSRC Sustainable Bioenergy Centre.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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