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Biochar–root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability

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Summary

Roots are the first point of contact between biochar particles and growing plants, yet detailed studies of biochar–root interactions are few. Biochar may affect root growth, and therefore plant performance, through two mechanisms: (i) as a direct nutrient source and (ii) through impacts on nutrient availability. To test the hypothesis that biochar–root interactions occur and are determined by biochar nutrient supply and impacts on soil nutrients, spring barley (*Hordeum vulgare* L.) was grown with and without biochar addition in rhizobox mesocosms. Biochar from unaltered and artificially weathered *Miscanthus* or willow (*Salix* sp) biochar types was used and was manipulated to alter its structure and nutrient content. After 28 days of plant growth, biochar nutrient content, soil nutrient content and the amount of biochar were measured in the bulk soil, the rhizosphere and the rhizosheath. Plants in biochar-amended soils had larger rhizosphere zones than the control treatment. The rhizosphere contained more biochar particles than the bulk soil, an indication that roots preferred soil containing biochar particles. Biochar particles retained soil nitrogen (N) in the form of nitrate, and also supplied phosphorus (P) to the soil and plant. *Miscanthus* biochar had a larger extractable P content than the *Salix* biochar, with different effects on plant growth and root responses. Although artificial physical weathering had no effect on overall plant growth, weathering effects on N retention and P content were dependent on biochar type. Our results indicate that roots are attracted towards biochar, resulting in its partitioning between bulk and rhizosphere soil. Biochar thus controls plant root nutrient acquisition directly as a nutrient source and indirectly by altering soil nutrient content.

Introduction

Roots are the first point of contact between biochar particles and plants; however, plant responses to biochar soil amendments have largely focused on above-ground biomass and crop yields. Root responses have generally been limited to biomass measurements in biochar studies (Lehmann *et al.*, 2003; Noguera *et al.*, 2010; Prendergast-Miller *et al.*, 2011). However, the mechanisms controlling root–biochar interactions are poorly understood (Lehmann *et al.*, 2011). Responses to biochar addition may be through a direct interaction between biochar particles and roots. Fine roots, root hairs or mycorrhizal fungal hyphae may take up nutrients, contaminants or water from surfaces or from internal biochar pores. Circumstantial evidence for such direct interactions arises from visual observation (Figure 1) of biochar particles clustered and bound to roots, root hairs or fungal hyphae (Joseph *et al.*, 2010; Lehmann *et al.*, 2011). Indirect biochar–root interactions could develop from: impacts on soil biogeochemistry (pH, nutrient availability, aeration or water holding capacity (WHC); Jones *et al.*, 2012); the structure and activity of the surrounding microbial community (Pietikäinen *et al.*, 2000; Rondon *et al.*, 2007); and release or sorption of chemical signals affecting root growth (Spokas *et al.*, 2010). These direct and indirect biochar–root interactions could initiate a range of responses in root systems and affect plant performance.

Root activity creates distinct root-soil zones. Thus biochar addition can affect root–soil interactions, for example, nitrate retention in the wheat (*Triticum aestivum* L.) rhizosphere of biochar-amended soils (Prendergast-Miller *et al.*, 2011). The rhizosheath is a layer of soil tightly bound to cereal roots. Root rhizosheath formation improves phosphorus (P) uptake under P-deficient conditions (Richardson *et al.*, 2009); however, the impacts of biochar on the rhizosheath have not been investigated. Plants growing in P-deficient soils develop thicker rhizosheaths because of longer root hair growth, which increases soil volume exploration and thus increases P uptake (Brown *et al.*, 2012).

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Figure 1 Direct interactions between woody biochar particles (*Acer* spp, Sycamore) and wheat roots facilitate nutrient and water uptake: fine roots and root hairs bind biochar particles within the rhizosheath (a); root hairs extend across biochar surfaces and within exposed biochar macropores (b–d). Biochar particles are approximately 2 mm long. Image credit © M. Prendergast-Miller.

Therefore, in a biochar-amended soil, rhizosheath development could indicate biochar-P availability, with a small rhizosheath indicating adequate to good P supply and a large rhizosheath indicating poor P conditions.

Most studies of the effects of biochar have used freshly produced materials, but biochar will age in soil and its function may alter. Fresh biochar can be manipulated in several ways to examine the effects of simulated ageing on biochar properties such as pH, cation exchange capacity (CEC) and nutrient content. This is achieved by heating (Cheng & Lehmann, 2009; Hale *et al.*, 2011), microbial degradation (Hale *et al.*, 2011), leaching to remove ash and labile compounds (Yao *et al.*, 2010), incubating in amended soil mixtures (Cheng *et al.*, 2006), physical crushing or grinding and oxidative chemical treatment. Soil containing naturally-aged biochar has also been used to determine ageing effects on N mineralization (Dempster *et al.*, 2012) and changes to its properties (Jones *et al.*, 2012). Longer-term changes (>100 years) in biochar pH and CEC can be inferred from studies of black carbon from historical charcoal sites (Cheng *et al.*, 2008). Comparing fresh and aged biochar products provides some indication of the longevity of biochar effects in soil, and whether the initial positive effects of biochar soil amendments can be sustained or are transient (Jones *et al.*, 2012) and so may require repeated biochar additions.

The aim of our experiment was to quantify biochar–root interactions. The effect of fresh (unaltered) and manipulated (artificially weathered) biochar on the growth of barley (*Hordeum vulgare* L.) was determined, with a focus on root characteristics. The nutrient content of bulk soil, the rhizosphere and the rhizosheath was measured to determine the effect of biochar on nutrient availability. The hypotheses were: (i) biochar–root interactions are determined directly by biochar nutrient content and indirectly through impact on soil nutrient availability; (ii) biochar particles retain soil nutrients, resulting in nutrient concentration in root-soil zones; and (iii) weathering biochar to alter nutrient content and effects on soil nutrient availability would determine whether nutrient content was an important factor in biochar-root interactions.

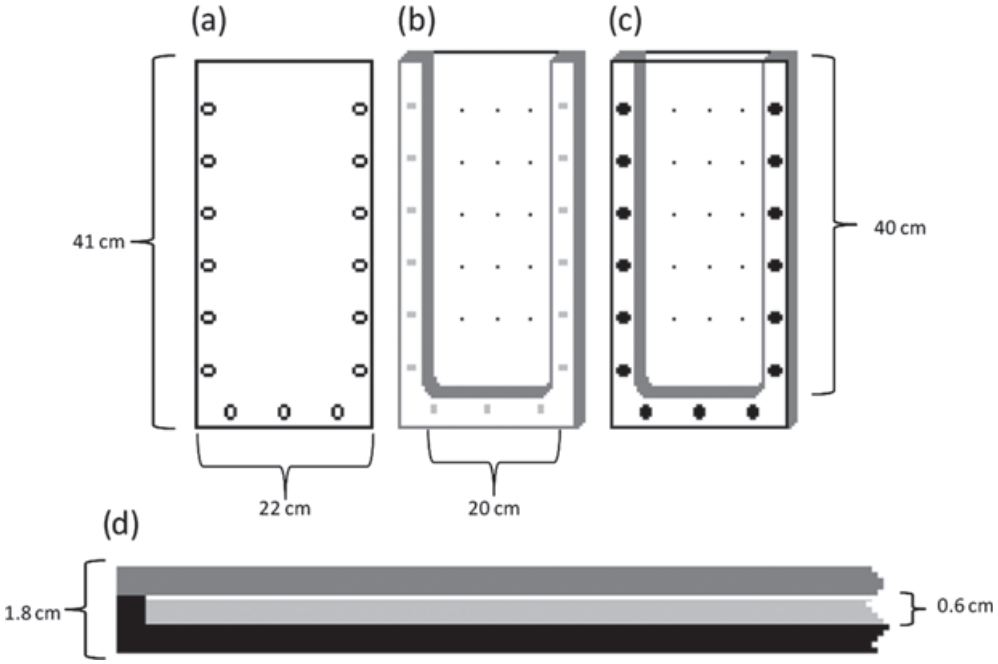

Figure 2 A schematic of a rhizobox mesocosm. Rhizoboxes were made from transparent Perspex sheets (0.6 cm thickness), with the front panel (a) held in place with screws and wing nuts. The back panel (b) with Perspex side walls had a grid of 3-mm diameter holes on the back face; (c) the assembled rhizobox; (d) a cross-section of the rhizobox indicates the thickness of the soil layer (0.5 cm, in pale grey) with a 1-mm air gap between the soil layer and the front face.

Materials and methods

Biochar

Biochar samples were created from two feedstocks: *Miscanthus* (*Miscanthus x giganteus*) straw (10–20-mm long) and willow (*Salix* sp.) wood chip (20–50-mm long). The *Miscanthus* biochar was produced in an auger-driven horizontal kiln at 700°C (Pyreg GmbH, Dörth, Germany) and the *Salix* biochar in a batch reactor at 450°C (J. Cook, School of GeoSciences, University of Edinburgh, Edinburgh, UK). The pyrolysis temperatures reflect the standard operating kiln temperatures of the pyrolysis units used. *Miscanthus* biochar was produced from straw and had the following characteristics: 82% C, 0.34% N, pH $9.58_{(water)}$, bulk density 0.13 g cm^{-3}, WHC 4.23 g g^{-1} and 9.08% ash content. *Salix* biochar was produced from wood chips and had 83% C, 0.91% N, pH $9.79_{(water)}$, bulk density 0.17 g cm^{-3}, WHC 2.54 g g^{-1} and 5.35% ash content.

Artificial weathering of biochar

The two biochar materials were crushed and sieved to collect approximately 200 g of a 1.0–2.0-mm fraction. Each sample was gradually brought to 60% WHC by spraying with deionized water. The samples were divided and one half covered and stored at room temperature (the non-weathered biochar). The other half of each sample was weathered artificially by subjecting it to a sequence of 200 controlled temperature oscillations between 30 and −10°C, with samples held at each temperature for 20 minutes (Thermotron 2800, Thermotron Industries, Holland, Michigan, USA).

Various properties of fresh and weathered samples were analysed to determine the degree of change to properties: pH in a suspension of 1:10 ratio in distilled water (Mettler Toledo FE20, Columbus, OH, USA), nitrogen, carbon as total, labile and stable C (Cross & Sohi, 2013), WHC, bulk density, and total and extractable nutrients. Total C and N were measured on milled samples (Carlo Erba 2500 Elemental Analyser, Rodano, Italy). Total P, K and Mg were determined using a modified dry-ashing method (Enders & Lehmann, 2012): milled biochar samples were heated for 8 hours at 500°C in a muffle furnace. Once cooled, 5 ml nitric acid was added and the samples placed over a steam bath until dry. A second aliquot of 5 ml nitric acid and hydrogen peroxide (1:5 ratio) was then added and the samples again steamed until dry. The samples were then solubilized in 65 ml deionized water, filtered (Whatman No. 42, GE Healthcare UK, Little Chalfont, Buckinghamshire, UK) and analysed for P, K and Mg (Perkin Elmer Optima 5300 DV ICP-OES, Waltham, MA, USA). Biochar extractable nutrients (NO_3-N, NH_4-N, P, K, Mg) were determined by using a serial water extraction (Angst & Sohi, 2013): biochar was mixed with deionized water (1:20 w/v) with octan-2-ol (1:200 octan-2-ol:water v/v) as a surfactant and shaken for 1 hour. The solution was filtered (Whatman No. 1, GE Healthcare UK) and the filtrate collected for analysis. The biochar was then dried at 50°C, and the extraction process repeated again a further four times, maintaining the 1:20 (biochar/water) and 1:200

Figure 3 Diagram showing soil collection from plant root systems: 'rhizosphere soil' (soil gently shaken free from plant roots) and the 'rhizosheath soil' (soil tightly adhering to plant roots and bound by root hairs; soil was separated from roots by shaking root systems in water). Soil remaining in the rhizobox was collected as the root-free 'bulk soil'. Image credit © M. Prendergast-Miller.

(octan-2-ol/water) ratios. Water soluble P, K and Mg were then measured by ICP (Perkin Elmer Optima 5300 DV ICP-OES) and inorganic N with an autoanalyser (Bran & Luebbe AA3, Seal Analytical, Norderstedt, Germany).

Scanning electron microscopy was used to assess any visual change to biochar structure. Images of unweathered biochar pieces were made with an electron microscope (XL 30 CP, Phillips, Eindhoven, The Netherlands) using secondary electron imaging at an accelerating voltage of 20 kV and a working distance of 10 mm. No gold coating was necessary because of the conductivity of the biochar. The orientation of the stubs within the microscope was marked and coordinates taken so that they could be placed back at the same orientation for subsequent imaging after weathering. Once imaged, the biochar on the stubs was subjected to the weathering process and returned to the electron microscope in the same position and orientation, allowing imaging and visual comparison with the previous non-weathered images.

Soil properties and biochar addition

A sandy loam soil from the Rothamsted Research experimental farm at Woburn, Bedfordshire (UK) was used. The soil (Stackyard series; Catt *et al.*, 1980) had the following characteristics: pH 5.8; mineral N content 23 mg kg^{-1}; available P content 5 mg kg^{-1}. The soil was not cultivated and had been maintained as a bare fallow for at least 40 years. Soil (47 kg dry weight) collected from the top 20 cm was sieved to < 2 mm and adjusted to 10% WHC with 1.48 g NH_4NO_3 in 550 ml distilled water to provide 11 mg N kg^{-1} soil. Soil (5 kg for each treatment) was then amended with one of the four biochar types: fresh *Miscanthus* biochar (M), weathered *Miscanthus* biochar (wM), fresh *Salix* biochar (S) or weathered *Salix* biochar (wS). Biochar was thoroughly mixed into the soil at 0.3% w/w (equivalent to a field application rate of 10 t ha^{-1} based on soil plough depth of 23 cm and bulk density of 1.58 g cm^{-3}). The control treatment had no added biochar.

Plant growth conditions

A rhizobox approach (Prendergast-Miller *et al.*, 2011) was used to investigate responses to fresh and weathered biochar samples. Soil from each treatment was packed to a soil density of 1.58 g cm^{-3} (soil thickness 0.5 cm) into transparent Perspex rhizoboxes (20 × 40 × 0.6 cm dimensions; Figure 2); there were six replicate boxes for each treatment. Each rhizobox received one pregerminated spring barley seedling (*Hordeum vulgare* L. var. Waggon): uniform seedlings with 20-mm radicles were selected. The rhizoboxes were then covered with aluminium foil to shield the roots from light. The rhizoboxes were placed at a 70° angle in a growth cabinet (Snijders Scientific Microclima Climate Chamber Model No. MC1000E, Tilburg, The Netherlands) at 20°C with a 16/8 hour day/night regime at 80% RH. Lighting (90 W m^{-2}) was provided by 12 × 36 W Brite Gro (Sylvania, Germany) fluorescent tubes (white) and 3 × 36 W Brite Gro tubes (red bias). The growing seedlings were watered to restore rhizobox mass every four days over a 28-day growth period. A grid of 3-mm diameter holes on the back face of each rhizobox allowed soil aeration, and the inner edges of the rhizobox were sealed with silicon to prevent water loss: we assumed that N losses as N_2O or leaching were therefore negligible.

Soil, biochar and plant analyses

At the end of the growth period, the rhizoboxes were dismantled and carefully sampled (Figure 3). The root systems were lifted from the soil and gently shaken to collect the 'rhizosphere' soil (soil adhering to roots) and weighed. For 'rhizosheath' soil (soil strongly held to roots), the roots with soil were weighed and placed in a 50-ml tube containing 15 ml distilled water and shaken by hand to remove the rhizosheath soil, which was collected as a sediment (Haling *et al.*, 2010); root mass was subtracted once quantified. The soil-free root samples were then rinsed over a fine mesh and stored in 20% ethanol. The root-free soil remaining in the rhizobox was collected as the 'bulk' soil and weighed.

The rhizosphere, rhizosheath and bulk soils were analysed for NH_4-N and NO_3-N in 1:10 w/v ratio 1 M KCl by autoanalyser. Soil pH in bulk and rhizosphere soil was measured in 1:10 w/v ratio in water (Mettler Toledo FE20), and rhizosheath pH was measured in the extracted sediment. Bulk and rhizosphere available P was

Table 1 Comparison of fresh and weathered biochar properties. Data shown are the mean of three replicates (SE)

Biochar property	*Miscanthus* biochar	Weathered *Miscanthus* biochar	*Salix* biochar	Weathered *Salix* biochar
Total C / %[a]	81.94	80.20	82.86	82.88
Total N / %[a]	0.34	0.32	0.91	0.90
$pH_{(1:10\ water)}$	9.70 (0.03)	9.78 (0.01)	9.42 (0.15)	9.29 (0.16)
Stable C / %[b]	98.16 (0.23)	99.79 (0.72)	96.58 (0.31)	97.16 (0.12)
Labile carbon / %[b]	0.25 (0.03)	0.23 (0.01)	0.13 (0.01)	0.13 (0.01)
Bulk density / $g\,cm^{-3}$	0.13 (0.002)	0.14 (0.003)	0.17 (0.004)	0.18 (0.004)
Water holding capacity / $g\,g^{-1}$	4.23 (0.11)	3.43 (0.01)	2.54 (0.2)	1.96 (0.02)
Extractable nutrients[c]				
P / $g\,P\,kg^{-1}$	0.27 (0.03)	0.22 (0.03)	0.04 (0.00)	0.04 (0.00)
NH_4 / $g\,N\,kg^{-1}$	0.001 (0.001)	0.001 (0.001)	0.003 (0.002)	0.002 (0.001)
NO_3 / $g\,N\,kg^{-1}$	0.007 (0.002)	0.007 (0.003)	0.003 (0.002)	0.003 (0.002)
K / $g\,K\,kg^{-1}$	11.59 (4.71)	8.49 (3.53)	1.04 (0.07)	1.31 (0.14)
Mg / $g\,Mg\,kg^{-1}$	0.02 (0.00)	0.02 (0.00)	0.06 (0.00)	0.07 (0.01)
Total nutrients[d]				
P / $g\,P\,kg^{-1}$	0.82 (0.13)	0.77 (0.17)	1.56 (0.16)	1.8 (0.34)
K / $g\,K\,kg^{-1}$	18.33 (0.39)	18.97 (0.34)	5.18 (0.15)	5.67 (0.11)
Mg / $g\,Mg\,kg^{-1}$	1.52 (0.04)	1.64 (0.04)	1.83 (0.04)	2.34 (0.04)
Na / $g\,Na\,kg^{-1}$	0.34 (0.006)	0.35 (0.009)	0.16 (0.01)	0.17 (0.01)

[a]Single measurements only.
[b]Methods given in Cross & Sohi (2013): stable and labile C are expressed as a proportion of total C.
[c]Extractable nutrients determined using a serial water extraction (Angst & Sohi, 2013).
[d]Total nutrients determined by modified dry ashing method (Enders & Lehmann, 2012).

measured in 2.5% acetic acid soil extracts (1:20 w/v ratio) and analysed by autoanalyser (Bran & Luebbe AA3, Seal Analytical, Norderstedt, Germany). All soil extract results are expressed on an oven-dry soil mass basis (dried at 105°C for 24 hour).

Biochar particles were separately recovered from three randomly selected rhizosphere and bulk soils per treatment, by hand with fine forceps. All visible biochar particles (> 0.5 mm) were collected from a 25 g (dry mass) subsample of rhizosphere or bulk soil, with any adhering soil particles removed. Biochar particles were analysed for NH_4^+-N and NO_3^--N by extracting with 1 M KCl and a sample mass to volume ratio of 1:100 (w/v). Extracts and blanks were filtered through 0.45-μm Millipore filters before analysis by autoanalyser.

Washed root samples were analysed for root characteristics using WinRHIZO Pro (2003b Regent Instruments, Quebec, Canada). Roots were rinsed and scanned in a tray of distilled water using an Epson 1670 desktop scanner at 600 dpi. The WinRHIZO data were used to calculate root traits (Prendergast-Miller *et al.*, 2011), morphology (root diameter classes) and topology (Fitter, 1987) (which quantifies root branching patterns). Root diameters were categorized as follows: main root axes (0.50–1.00 mm), first order lateral roots (0.25–0.50 mm) and secondary fine roots (< 0.25 mm). The WinRHIZO program algorithm was used to measure root magnitude (the number of external links in a root system, μ) and altitude (the number of links in the longest path length, a) of root axes for each root system, which were then used to determine the root system topological index (TI) of individual rhizoboxes (Equation (1)):

$$TI = \text{slope } (\log\ a) / \text{slope } (\log\ \mu) \quad (1)$$

The topological index describes the extent of root branching: a TI value close to 1 describes a 'herringbone' structure (less branching, where the system is restricted to a main axis and primary laterals). A TI value close to 0.5 describes a 'dichotomous' pattern (where the system is more complex and highly branched) (Fitter, 1987). After scanning, roots were dried at 70°C for 48 hours for dry mass determination. Root samples were then milled and analysed for total N (Carlo Erba 2500 Elemental Analyser, Rodano, Italy).

H. vulgare leaves were collected and dried at 70°C for 48 hours for dry mass determination; leaf samples were then milled and analysed for total N (Carlo Erba 2500). Total phosphorus was determined with a wet digestion method with concentrated H_2SO_4 and 30% H_2O_2; extracts were then analysed on the autoanalyser.

Data analysis

Data were tested for normality and homogeneity of variance. Data for root N and P uptake were log-transformed before use. Differences between treatments and within biochar types were tested using ANOVAs with Minitab 14 (Minitab Inc., State College,

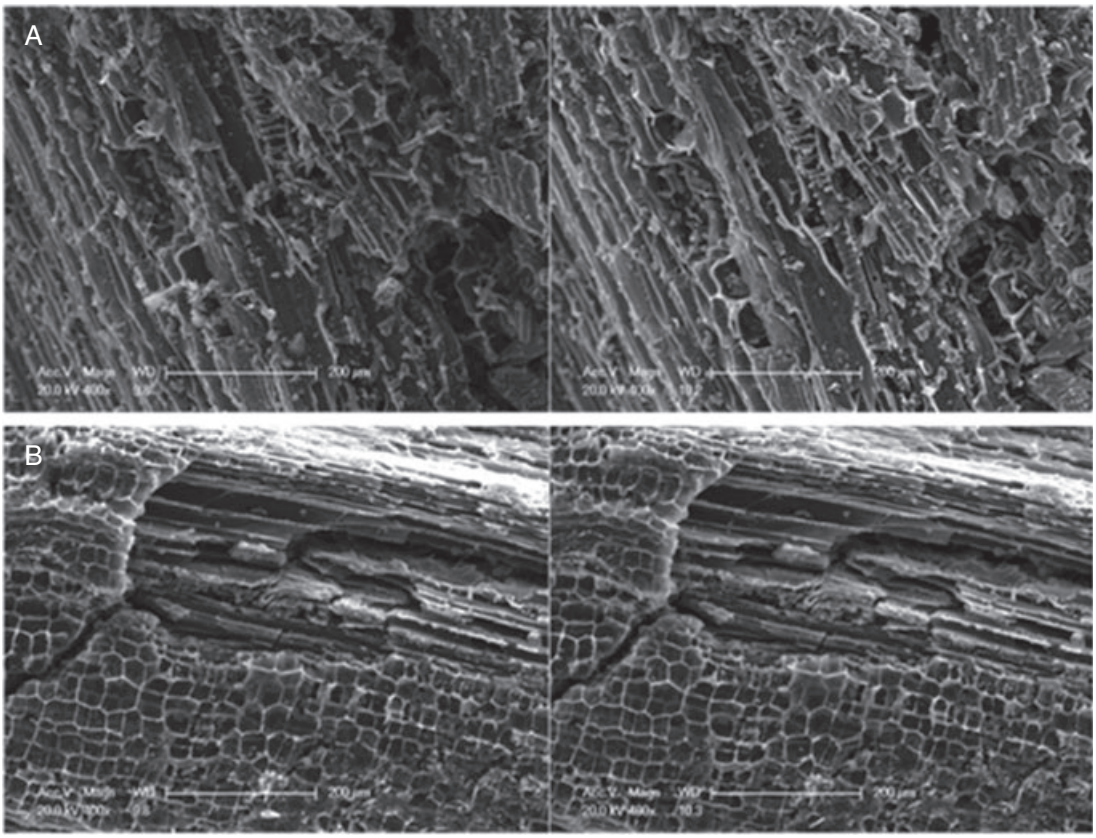

Figure 4 Scanning electron micrographs of the same piece of biochar before (left) and after (right) artificial weathering of *Miscanthus* biochar (a) and *Salix* biochar (b). The weathering treatment produced slight changes to biochar surfaces, indicating no major structural alterations. Image credit © M. Duvall.

PA, USA). Non-parametric tests were used where indicated when transformation failed to improve the distribution. Reported data show mean ± standard error (SE).

Results

Biochar properties and effect of simulated weathering

The fresh *Miscanthus* and *Salix* biochar types were broadly similar in terms of C, pH, bulk density and WHC: although some properties were statistically significantly different ($P < 0.05$), the magnitude was small (see Table 1). Both biochar types were highly stable with a small labile C content (< 1% of total C). Greater differences were present in the nutrient content: the *Salix* biochar had a larger total P ($1.56 \pm 0.16\,g\,P\,kg^{-1}$) and Mg ($1.83 \pm 0.04\,g\,Mg\,kg^{-1}$) content, while *Miscanthus* biochar had a larger K ($18.33 \pm 0.39\,g\,K\,kg^{-1}$) and Na ($0.34 \pm 0.006\,g\,Na\,kg^{-1}$) content. However, in terms of available biochar nutrients, more P was water-extractable from *Miscanthus* biochar ($0.27 \pm 0.03\,g\,P\,kg^{-1}$), which represented 32% of total P ($0.82\,g\,P\,kg^{-1}$), than from *Salix* biochar, where only $0.04 \pm 0.004\,g\,P\,kg^{-1}$ was water extractable, which represented 2.7% of total P ($1.56\,g\,P\,kg^{-1}$; see Table 1). Simulated weathering resulted in no dramatic change to most measured properties of either *Miscanthus* or *Salix* biochar. There were significant but small differences in changes to soluble K, WHC and bulk density. Scanning electron microscopy of biochar particles before and after weathering showed no visual evidence of notable structural change in biochar structure (see Figure 4).

Effect of biochar types on post-harvest soil properties

Biochar altered soil N at the start of the experiment ($P < 0.05$): soil mineral N contents were 34 ± 1.46, 61 ± 6.80, 83 ± 17.19, 61 ± 16.2 and $33 \pm 7.45\,mg\,N\,kg^{-1}$ in the control, *Miscanthus*, weathered *Miscanthus*, *Salix* and weathered *Salix* treatments, respectively. Biochar increased mineral N by a factor of two in the *Miscanthus* and *Salix* treatments; however, the weathered *Salix* biochar was similar to the control. At harvest, the concentration and form of soil mineral N differed between the bulk, rhizosphere and rhizosheath soils. Ammonium content (Figure 5a) was different between soil zones across all treatments ($P < 0.001$). Although soil NH_4^+-N content was less than NO_3^--N, NH_4^+-N tended to increase closer to plant roots and was greatest in the rhizosheath for the control treatment ($5.5\,mg\,N\,kg^{-1}$). There was no effect of biochar addition ($P > 0.05$) or significant interaction ($P > 0.05$). Bulk soil NO_3^--N in unweathered and weathered

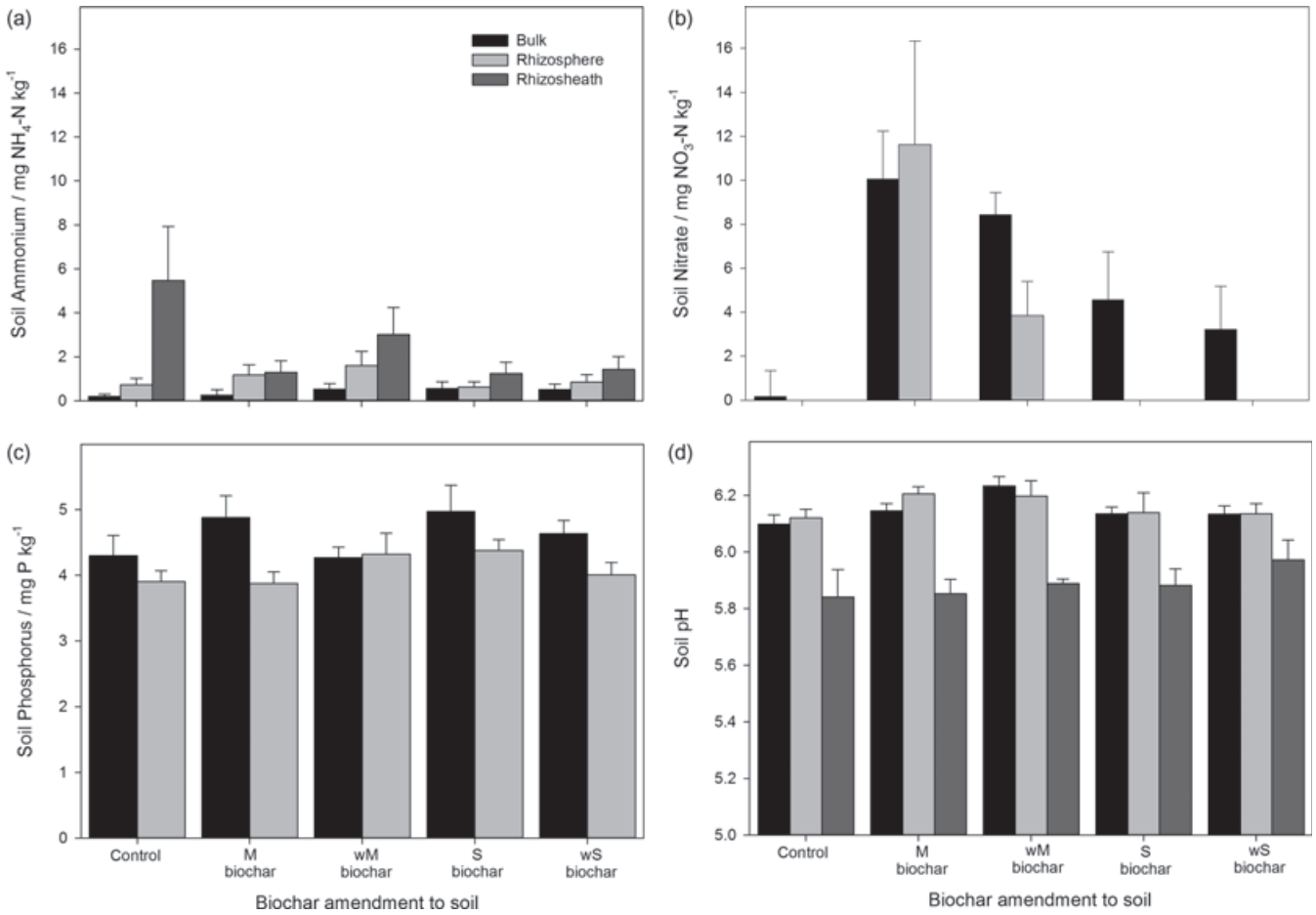

Figure 5 Ammonium (a), nitrate (b), phosphorus (c) content and pH (d) of soils with and without biochar measured from bulk, rhizosphere and rhizosheath soils, after 28 days plant growth. Treatments were control (soil only, no biochar) and soil amended with one of four biochar types: *Miscanthus* biochar (M), weathered *Miscanthus* biochar (wM), *Salix* biochar (S) and weathered *Salix* biochar (wS). Error bars show + SE ($n = 6$).

biochar treatments was approximately nine (*Miscanthus*) and three (*Salix*) times greater ($P < 0.001$) than in the control treatment. In the rhizosphere, NO_3^--N was only detected in *Miscanthus* biochar treatments (11.6 and 3.8 mg NO_3^--N kg^{-1} in the rhizosphere of unweathered and weathered treatments, respectively), but there was no significant weathering effect ($P > 0.05$). No NO_3^--N was detected in the rhizosheath of control or any of the biochar-amended soils (Figure 5b).

Adding fresh *Miscanthus* or *Salix* biochar to soil increased ($P < 0.001$) initial soil P contents by about 0.6 mg P kg^{-1} while adding weathered biochar made no change (Figure 5c). At harvest, bulk soil P tended to be larger in treatments with fresh biochar. Overall, rhizosphere P content was generally less than in the bulk soil ($P < 0.01$), except for weathered *Miscanthus*, where rhizosphere and bulk soil P were the same. Although *Miscanthus* biochar tended to increase soil pH slightly in the bulk and rhizosphere zones, the trend was non-significant ($P > 0.05$; Figure 5d), except in the bulk soil, where weathered *Miscanthus* biochar increased pH by 0.2 units ($P < 0.05$). Across all treatments, bulk and rhizosphere soil were similar in pH, although it was lowest in the rhizosheath ($P < 0.001$).

Shoot and root characteristics

Shoot and root analyses are presented in Tables 2 and 3 (with ANOVA information given in Tables S1, S2). Biochar addition increased both shoot ($P < 0.01$) and root ($P < 0.01$) biomass, with the greatest increase in the *Miscanthus* biochar treatment. Total root length was not affected by biochar treatment. Plants growing in the *Miscanthus* treatments had a smaller shoot N content (21 mg g^{-1}) than the control and *Salix* treatments (27 mg g^{-1}) ($P < 0.05$), but there was no treatment effect on shoot N accumulation or root N uptake. However, shoot P content ($P < 0.01$) and accumulation ($P < 0.001$) and root uptake ($P < 0.05$) were greater in the *Miscanthus* biochar treatments. Plants growing in the *Miscanthus* biochar treatments were within the normal range for P content (0.30–0.50% P), while those in the control and *Salix* biochar treatments had P contents that were smaller (0.24–0.29% P) but more than P deficiency (< 0.24% P)

Table 2 Plant shoot analyses from biochar-amended soils using fresh or weathered *Miscanthus* and *Salix* biochar types. Data are the means of six replicates per treatment (SE) (see Table S1 for ANOVA summary)

Plant property	Control	*Miscanthus* biochar	Weathered *Miscanthus* biochar	*Salix* biochar	Weathered *Salix* biochar
Shoot biomass / g dry mass	0.15 (0.01)	0.19 (0.003)	0.18 (0.01)	0.15 (0.01)	0.13 (0.01)
Shoot N content / mg N g^{-1} shoot	26.4 (1.55)	21.3 (0.41)	22.8 (1.5)	26.9 (2.16)	28.6 (1.77)
Shoot P content / mg P g^{-1} shoot	2.60 (0.16)	3.28 (0.21)	3.13 (0.17)	2.53 (0.16)	2.51 (0.14)
N uptake / mg N $shoot^{-1}$	3.88 (0.07)	4.08 (0.13)	3.93 (0.11)	3.77 (0.15)	3.82 (0.07)
P uptake / mg P $shoot^{-1}$	0.38 (0.02)	0.63 (0.04)	0.55 (0.06)	0.34 (0.03)	0.37 (0.04)

Table 3 Plant root analyses from biochar-amended soils using fresh or weathered *Miscanthus* and *Salix* biochar types. Data are the means of six replicates per treatment (SE) (see Table S2 for ANOVA summary)

Plant trait	Control	*Miscanthus* biochar	Weathered *Miscanthus* biochar	*Salix* biochar	Weathered *Salix* biochar
Root biomass / g dry mass	0.10 (0.008)	0.14 (0.008)	0.12 (0.01)	0.10 (0.006)	0.08 (0.007)
Root N content / mg g^{-1}	8.33 (0.74)	7.69 (0.25)	8.18 (0.30)	8.59 (0.55)	9.22 (0.50)
Total root length / cm	1117.1 (113.3)	1345.4 (155.0)	1305.1 (110.5)	1013.9 (132.0)	1117.5 (158.2)
Specific root length / cm root mg^{-1} root	11.18 (1.28)	9.7 (11.1)	10.91 (0.42)	9.85 (1.45)	13.73 (1.70)
Root weight ratio / g root g^{-1} plant	0.40 (0.01)	0.42 (0.02)	0.40 (0.01)	0.42 (0.03)	0.38 (0.01)
Root length ratio / cm root mg^{-1} plant	4.51 (0.52)	4.06 (0.45)	4.40 (0.10)	4.03 (0.49)	5.13 (0.55)
Root N uptake / mg shoot N cm^{-1} root	0.10 (0.02)	0.07 (0.01)	0.07 (0.01)	0.12 (0.02)	0.11 (0.02)
Root P uptake / mg shoot P cm^{-1} root	0.36 (0.03)	0.49 (0.05)	0.42 (0.02)	0.39 (0.06)	0.32 (0.04)
Log magnitude	2.23 (0.18)	2.35 (0.19)	2.1 (0.25)	2.25 (0.3)	1.75 (0.21)
Log altitude	1.85 (0.11)	1.73 (0.17)	1.68 (0.19)	1.82 (0.15)	1.60 (0.19)
Topological index	0.60 (0.08)	0.60 (0.06)	0.50 (0.14)	0.60 (0.04)	0.50 (0.13)
Rhizosphere soil / g dry mass $rhizobox^{-1}$	141.8 (18.8)	164.5 (15.1)	151.7 (7.9)	106.3 (10.3)	122.3 (4.8)
Rhizosheath soil / g dry mass $rhizobox^{-1}$	43.2 (3.80)	39.0 (3.70)	36.1 (5.10)	36.1 (3.30)	31.8 (2.40)

(Weir & Cresswell, 1994). Plants in all treatments were N deficient (< 3.4% N) (Weir & Cresswell, 1994). The increase in biomass was linked to soil P availability as uptake was correlated with root length ($P < 0.001$).

The presence of *Miscanthus* biochar (fresh or weathered) tended to result in reduced specific root length and root length ratio compared with the control, while root traits for plants growing in fresh or weathered *Salix* biochar types were generally similar to plants growing without biochar (Table 3). The topological data (Equation (1)) showed that all plants had highly branched root systems. Plants growing in weathered biochar-amended soils appeared to have less branched root patterns (TI of 0.5) than those in the control and unweathered biochar treatments (TI of 0.6), but these differences were non-significant ($P > 0.05$). Root diameter distribution (Figure 6) indicated that lateral and fine roots contributed the most to total root lengths in all treatments, and the greater root production between control and *Miscanthus* biochar was consistent between root diameter classes ($P < 0.05$).

Plants growing in the fresh or weathered *Miscanthus* biochar treatments had the largest rhizospheres (164 and 151 g dry mass respectively) (Table 3). In the control treatment, the rhizosphere (142 g dry mass) was approximately three times greater than the rhizosheath (43 g dry mass) (see Table 3). In the *Miscanthus* biochar treatments, regardless of type, the rhizosphere was four times greater than the rhizosheath ($P < 0.05$). The rhizosphere (114 g dry mass) and rhizosheath (34 g dry mass) were smallest in the *Salix* biochar treatments. Although rhizosheath dry masses tended to be smaller in the biochar treatments, this was not significant (Table 3). However, rhizosheath development, measured using a rhizosheath mass:root length ratio (Haling *et al.*, 2010), indicated that plants growing in soil amended with *Miscanthus* biochar had smaller rhizosheaths than the control ($P < 0.05$; Figure 7).

Quantification of biochar in soil

Biochar particles were recovered from bulk and rhizosphere soils to establish any association of roots with biochar particles. Clear differences emerged between these zones (Figure 8a). For both biochar types, biochar content was greater ($P < 0.001$) in the rhizosphere (3.24 ± 0.06 and 3.79 ± 0.16 mg g^{-1} soil for *Miscanthus* and *Salix*, respectively) than in the bulk soil (2.75 ± 0.09 and 3.08 ± 0.11 mg g^{-1} soil for *Miscanthus* and *Salix*, respectively). The difference was greater ($P < 0.01$) in the *Salix* treatments than in the *Miscanthus*, and weathering had no effect. Similarly, there was an effect of biochar type on the ratio of biochar mass to root mass (Figure 8b), with *Salix* biochar

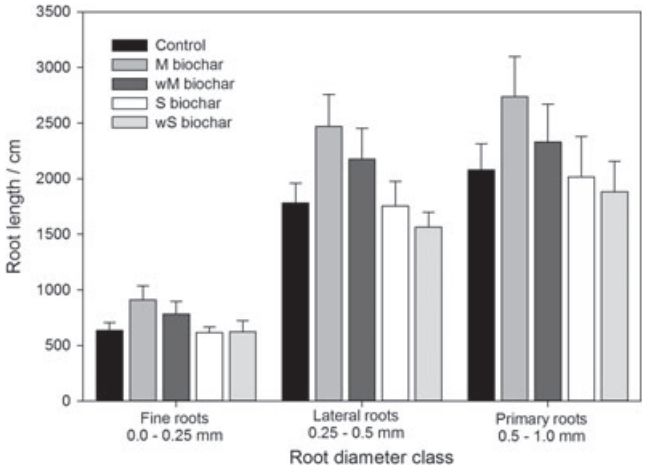

Figure 6 Root lengths and diameter classes across treatments (control, no biochar; soil + *Miscanthus* biochar (M); soil + weathered *Miscanthus* biochar (wM); soil + *Salix* biochar; soil + weathered *Salix* biochar (wS)). Error bars show + SE ($n = 6$).

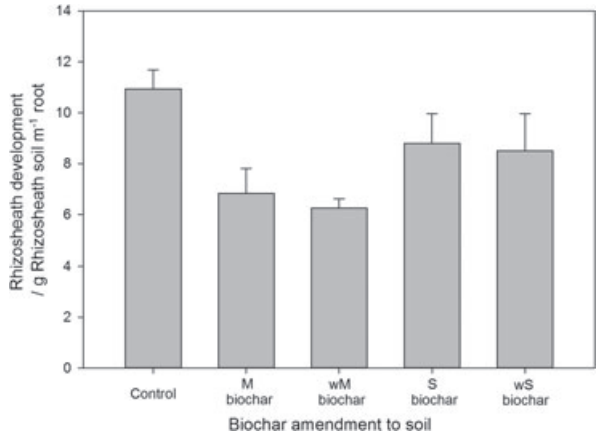

Figure 7 Ratio of rhizosheath mass: root length indicating development of the rhizosheath in different biochar treatments (control, no biochar; soil + *Miscanthus* biochar (M); soil + weathered *Miscanthus* biochar (wM); soil + *Salix* biochar; soil + weathered *Salix* biochar (wS)). Error bars show + SE ($n = 6$).

having the greater effect ($P < 0.05$): there was also no effect of weathering.

Quantification of N in biochar particles

Nitrate was the dominant form of N extracted from biochar particles. Biochar-associated N was partitioned in different soil zones, which varied with biochar type (Figure 9a,b). Approximately 6 and 4 $\mu g\,N\,g^{-1}$ soil was measured in *Miscanthus* biochar particles retrieved from bulk and rhizosphere samples, respectively; weathering reduced the amount of biochar-N to about 3.5 $\mu g\,N\,g^{-1}$ soil but the content was similar in the bulk and rhizosphere soil. Biochar-associated N content was an order of magnitude smaller with *Miscanthus* than with fresh *Salix* biochar

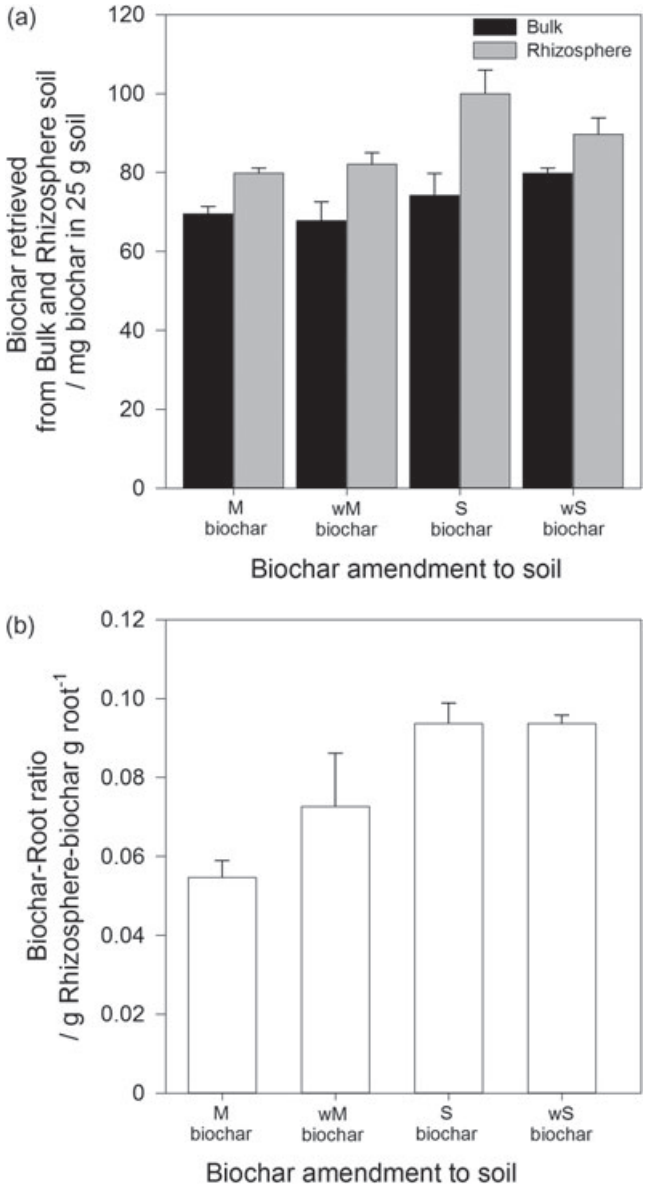

Figure 8 Fresh and weathered biochar retrieved from bulk and rhizosphere soil subsamples (a). Rhizosphere ratios compare the rhizosphere biochar weight with root weight (b). Error bars show + SE ($n = 3$).

(about 0.05 $\mu g\,N\,g^{-1}$ soil) but increased to about 0.12 $\mu g\,N\,g^{-1}$ soil in the rhizosphere of weathered *Salix* biochar. Biochar-N in the *Salix* bulk soil was not affected by weathering.

Miscanthus biochar accounted for one-quarter of total mineral N in the rhizosphere and one-half in the bulk soil (Table 4). This trend was consistent between fresh and weathered *Miscanthus* biochar. Although the concentration of mineral N was less in the *Salix* biochar than in *Miscanthus* (Figure 9b), relatively more N was held in rhizosphere *Salix* biochar. This trend increased with weathering. However, *Salix* biochar-N was a small proportion of soil mineral N, indicating that relatively little N was retained in *Salix* biochar types at the end of the 28-day plant growth period.

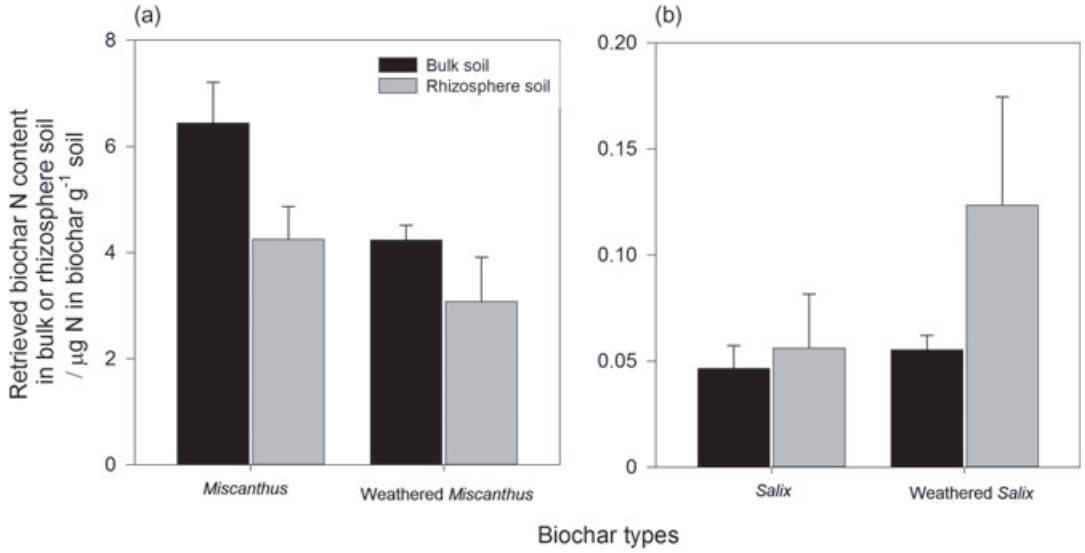

Figure 9 The amount of N extracted from fresh and weathered *Miscanthus* and *Salix* biochar types from bulk and rhizosphere soils (a, b, respectively). Error bars show + SE ($n = 3$).

Table 4 Soil N partitioning: the amount of soil-extractable and biochar-extractable N from biochar-amended soils using fresh or weathered *Miscanthus* and *Salix* biochar types

Treatment	Bulk soil N / µg N g^{-1} soil	Rhizosphere soil N / µg N g^{-1} soil	Biochar-N in bulk soil / µg N g^{-1} soil	Biochar-N in rhizosphere soil / µg N g^{-1} soil
Control	1.28 (0.79)	0.76 (0.42)	–	–
Miscanthus biochar	10.47 (2.28)	12.77 (3.35)	6.44 (0.77)	4.25 (0.62)
Weathered *Miscanthus* biochar	9.00 (1.14)	5.46 (1.42)	4.23 (0.28)	3.07 (0.84)
Salix biochar	5.44 (1.92)	0.62 (0.11)	0.05 (0.01)	0.06 (0.03)
Weathered *Salix* biochar	4.17 (1.68)	0.88 (0.32)	0.06 (0.06)	0.12 (0.05)

Nitrogen budget

Figure 10 shows the quantity of mineral N in each measured pool (plant (shoot plus root), biochar and soil zone). Results are expressed as a proportion of rhizobox mineral N at the start of the experiment after fertilizer (11 mg N kg^{-1} soil) and biochar amendment. The small amount of N introduced in the biochar amendments (Table 1) was assumed to be negligible as it is recalcitrant (Knicker, 2010). The budget shows that biochar maintained larger mineral N concentrations in the bulk and rhizosphere soils than in the control, with greater differential partitioning of N between soil and biochar for *Miscanthus*-derived material than for the *Salix* biochar. Importantly, this biochar N retention did not affect plant N uptake, which was similar across treatments (Table 2). The proportion of biochar-associated mineral N varied with biochar type: the *Miscanthus* biochar retained more N than the *Salix* biochar, and this was decreased by weathering. *Miscanthus* biochar increased N partitioning into the rhizosphere but weathering appeared to reduce this effect. In the *Salix* biochar treatment, biochar-N retention was minimal (Table 4). The remaining N has been termed 'immobilized N' as microbial biomass N was not independently measured. Rhizosheath biochar-N was also not quantified but is likely to form a smaller pool than the microbial pool. The larger 'immobilized N' pool in the control and *Salix* biochar treatments suggests that these treatments may have been nutrient limited.

Discussion

Effects of artificial weathering on biochar properties

Manipulating *Miscanthus* and *Salix* biochar using an artificial weathering approach did not change pH, total C and N, C stability or overall nutrient content, which is similar to results reported by Hale *et al.* (2011), who applied a freeze-thaw physical weathering treatment but at more extreme temperature ranges (−70 to 20°C) over a 2-month period. Under our experimental conditions, there were small significant changes to WHC (decreased by 0.5–0.8 g g^{-1}), bulk density (increased by 0.01 g cm^{-3}), K release (decreased by 3.0 g K kg^{-1} with *Miscanthus* but increased by 0.3 g K kg^{-1} with *Salix*) and total Mg content (increased in the *Salix* treatment by 0.5 g Mg kg^{-1}) (Table 1). These changes could have resulted from pores collapsing or clogging that affected accessibility but only to a minor degree.

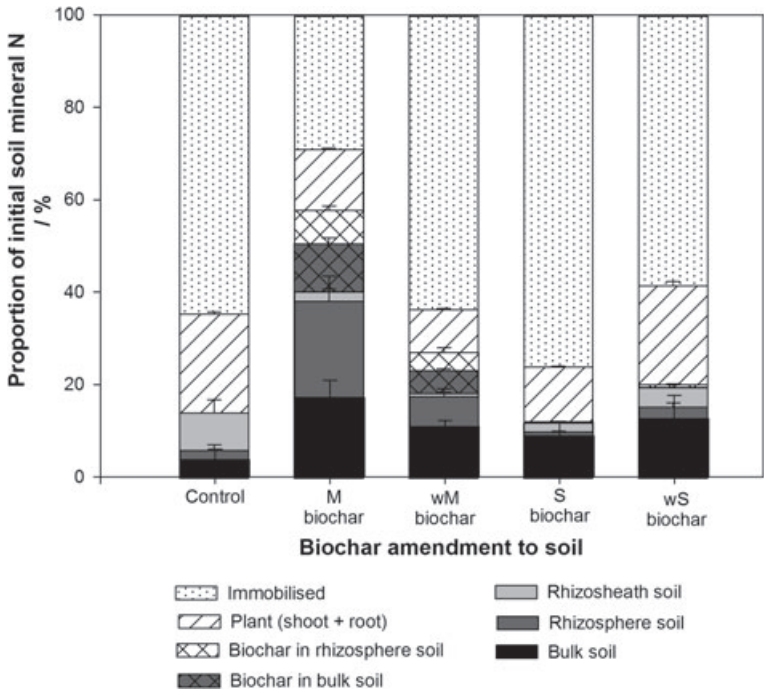

Figure 10 The fate of mineral N in the rhizoboxes after 28 days of plant growth from different biochar treatments. 100% indicates the initial soil mineral N for each treatment: control 34.7, *Miscanthus* 60.8, weathered *Miscanthus* 82.6, *Salix* 61.1 and weathered *Salix* 33.0 mg N kg^{-1}. Open bars show the mineral N pool, which was probably immobilized by the microbial biomass, as well as unmeasured N (rhizosheath soil biochar N). Loss of N by leaching and N_2O was assumed to be negligible under the given experimental conditions. *Salix* biochar N content was 0.06 mg N kg^{-1} in both bulk and rhizosphere zones; weathered *Salix* biochar N content was 0.06 and 0.12 mg N kg^{-1} in bulk and rhizosphere zones, respectively.

The lack of a stronger impact of the manipulation on initial biochar properties in Table 1 might be explained by the shorter weathering time, and hence fewer cycles (192 hour) than in other studies of 300 hours (Yao *et al.*, 2010), 2 months (Hale *et al.*, 2011) or 6–12 months (Cheng & Lehmann, 2009). The type of manipulation may also be important, as simulated physical weathering (freeze-thaw treatments) applied by Hale *et al.* (2011) had less dramatic changes than heating or biological treatment. It is also likely that the rate of weathering and its ability to affect the interior biochar structure would be relatively slower under our experimental conditions than with the wider temperature ranges used by others (Cheng & Lehmann, 2009; Hale *et al.*, 2011). Although the intrinsic differences between *Miscanthus* and *Salix* biochars remained greater than any change brought about by artificial weathering, there was a difference between fresh and weathered biochar with N retention and biochar–root interactions. At a microscale, the measured changes to WHC and bulk density could affect root hair and pore-water dynamics not captured by our methods.

Biochar-associated nutrients

Miscanthus biochar had a greater extractable P content than that of *Salix* and although the total P content of *Salix* biochar (1.56 g P kg^{-1}) was almost double that of *Miscanthus* (0.82 g P kg^{-1}), the majority was insoluble or inaccessible as only 2.7% could be removed by extraction. Thus it is likely that *Salix* biochar provided less available P than *Miscanthus* biochar. This was clearly demonstrated by the increased shoot P content and root growth responses in the *Miscanthus* biochar treatment. Both *Miscanthus* and *Salix* biochar had small N contents and negligible extractable N. Mineral N tends to be the less available biochar-associated nutrient (Table 1) (Glaser *et al.*, 2002; Lehmann *et al.*, 2003). Although biochar may not be an initial direct source of N, our data show that soil-recovered biochar retains mineral N, mainly as NO_3^--N: this discrete biochar-N pool was up to one-quarter or one-half of soil mineral N, depending on whether it was from bulk or rhizosphere soil and biochar type (being more evident for *Miscanthus*- than *Salix*-derived biochar). Weathering decreased N retention in both the bulk soil and the rhizosphere in the *Miscanthus* treatment. Although less N was retained by the *Salix* biochar, weathering increased retention in the biochar recovered from rhizosphere soil (Figure 9). There are few reports of retrieval of biochar particles to measure biochar-N (Prendergast-Miller *et al.*, 2011; Jones *et al.*, 2012), but they show greater biochar- NO_3^--N content than unmanipulated or fresh material. These data suggest that N was held in solution in biochar pores: an increase in NO_3^--N can be attributed to increased water retention by biochar (Glaser *et al.*, 2002). The difference in biochar-N retention between unweathered and weathered *Miscanthus* and *Salix* biochar (Figure 9) may reflect differences in internal biochar pore structure related to feedstock.

Biochar effects on soil nutrients

In the control treatment, NH_4^+-N concentrations increased closer to the root and were least in bulk soil, which is similar to Herman *et al.* (2006). However, in biochar treatments, smaller NH_4^+-N concentrations in rhizosheath soils suggest greater NH_4^+-N uptake or less N mineralization. Noguera *et al.* (2010) reported increased mineral N content in a rice-biochar experiment; as in our study, NO_3^--N content was greater than NH_4^+-N and most of the

soil N measured was as NO_3^--N (Figure 5b), suggesting that nitrification occurred in biochar-amended soils. It is possible that some mineralization of organic matter occurred with addition of labile biochar-C; however, this would have been only a small contribution because of poor lability (Cross & Sohi, 2011). Nitrification within the rhizosphere can be stimulated (Norton & Firestone, 1996) or inhibited (Herman *et al.*, 2006) and biochar studies that investigate N transformations within soil zones most influenced by the plant (rhizosphere and rhizosheath) are required.

Plants growing in the control and *Salix* biochar treatments had greater shoot N content and smaller root-zone mineral N than *Miscanthus* treatments, which had greater rhizosphere and bulk soil N but smaller shoot N. However, as shoot N uptake was similar for all treatments (Table 2), and plants in all treatments showed N deficiency (Weir & Cresswell, 1994), this suggests that biochar and biochar-N retention did not adversely affect N availability.

Root responses to biochar

We found that quantifying biochar particles in the rhizosphere and rhizosheath development were more useful indicators of root behaviour than root trait measurements. Quantification of biochar particles within different soil zones demonstrated that the rhizosphere contained more biochar than the bulk soil (Figure 8). As biochar was not mobile in the rhizoboxes, we conclude that roots grow preferentially towards biochar, and in so doing, the rhizosphere is extended. As biochar-amended bulk soils had greater N contents than the control (Figure 5b), it is possible that with continued plant growth, the rhizosphere would extend to capture the N held in bulk soils. Slightly more biochar was retrieved from both fresh and weathered *Salix* rhizosphere soil (Figure 8b), suggesting that another biochar-associated nutrient such as Mg (see Table 2) may have been important for plant growth, as *Salix* biochar had a small extractable P content. Therefore, differences in pore size and structure, extractable nutrient contents and solute N retention capabilities between *Salix* and *Miscanthus* biochar types may regulate root responses to biochar.

Measurements of rhizosheath development, which is related to root hair length (Haling *et al.*, 2010), have indicated an important mechanism whereby biochar-root interactions are mediated at the microscale of root hairs and biochar pores (Figure 1). Root systems in the control treatment had larger rhizosheaths (11 g m^{-1}; Figure 7) than biochar treatments (6 and 8 g m^{-1} for *Miscanthus* and *Salix*, respectively). Large rhizosheaths are associated with longer and denser root hairs, which facilitate P uptake under poor P conditions (Richardson *et al.*, 2009; Brown *et al.*, 2012). It is likely that the control treatment was P-limited, resulting in the large rhizosheath. In contrast, the biochar treatments received some additional biochar-P and so developed smaller rhizosheaths than the control. As more P was available from *Miscanthus* biochar (Table 1), plants in this treatment had the smallest rhizosheath (Figure 7).

Conclusions

Plant roots respond to soil amendment because biochar can serve as a direct nutrient supply through addition of soluble P and biochar-N retention, as well as the indirect effects of biochar on soil nutrient availability through increased N retention in the rhizosphere and bulk soils. Evidence of these direct and indirect interactions was shown by the larger rhizosphere in biochar-amended soils; the rhizosphere contained more biochar particles; and rhizosheath development was affected by biochar-P availability. Therefore, we conclude that roots respond to biochar particles and that this is controlled by nutrient and possibly water availability. As well as increased soil N retention in bulk and rhizosphere soil zones, biochar particles can retain a significant proportion of soil-extractable nitrate-N. Artificial weathering altered some properties of biochar, which subsequently affected biochar-N retention and rhizosheath development, which indicates that biochar nutrient content is an important factor in biochar-root interactions. Differences in nutrient concentrations from plant-soil zones as well as from biochar particles demonstrate the benefits of studies conducted at relevant scales, rather than analyses of bulk soil samples. Understanding the relationships between biochar and soil pores, and sorption and release of water and associated soluble nutrients at the scale of biochar particles is now necessary to quantify and predict biochar function in soil. Addition of a porous biochar may affect soil pore connectivity; therefore, detailed microsite measurements are required to determine the mechanisms that enable biochar to mediate nutrient availability to roots.

Supporting Information

The following supporting information is available in the online version of this article:

Table S1: ANOVA summary table for plant shoot analyses given in Table 2.

Table S2: ANOVA summary table for plant root analyses given in Table 3.

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