

**Report on the effect of fresh and weathered biochar on the architecture of
crop roots and soil mineral nitrogen in the rhizosphere**

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This report has been put together for the Interreg IVB project: Biochar: climate saving soils
The objectives for this report were:

Objective 4.2.1: Systems to screen biochar–plant interactions

- The effect of biochar on root architecture and the localisation of soil mineral nitrogen in the rhizosphere, characterized using mesocosms.

Objective 4.2.2: Testing the simulation of physical ageing of biochar in the field

- The effect of time on the effects of biochar on the rhizosphere, assessed by simulated (physical) ageing.

Introduction

The potential for biochar to positively affect crop growth are various, through effects on a range of chemical and physical phenomena in soil, that in turn impact on biological processes in the plant, soil and rhizosphere (root zone).

Since the different biochar materials are diverse in their physical and chemical properties, these being determined by a particular combination of feedstock and conversion (pyrolysis) parameters, there is a need to develop some generalisations or 'rules of thumb', in order to predict how certain types of biochar will impact certain soil–crop combinations – and to select biochar that is beneficial.

An impact of biochar on the cycling of soil nitrogen is increasingly recognised. Plants require nitrogen to grow. In agriculture it is the key annual input in terms of fertiliser for crop nutrition, inorganic or organic. Biochar does not supply nitrogen – it is one of the few elements that is volatile at pyrolysis temperatures and eliminated (as gas), or integrated with C into the stable molecular structures that predominate in biochar.

However, biochar interacts with mineral nitrogen that is already in the soil, or mineral nitrogen that becomes available through mineralisation of organic matter. Soil mineral nitrogen accounts for only a few per cent of total soil nitrogen but is the form that plant roots can access. Its status depends on the dynamic balance between mineralisation, plant uptake and its utilisation by micro-organisms. As well as proliferation or replacement of biomass (immobilisation), micro-organisms that use nitrogen (rather than carbon) as electron acceptors, can oxidise mineral nitrogen to the potent greenhouse gas nitrous oxide.

The mineral nitrogen pool itself comprises two key components: soluble nitrate (an anion), and the ammonium cation. Nitrate is the main source of leached nitrogen, nitrous oxide and crop nutrition. Nitrate is a product of microbial conversion of ammonium and the sometimes apparent effect of biochar on nitrous oxide emissions and leaching (both can be decreased), has usually been attributed to the indirect effect on ammonium sorption. Biochar tends to have a negative charge and thus exchange sites for cations. Depending on the rate of biochar application, a decrease in soil nitrate concentrations can be partially explained by the capture of ammonium. However, the impact of ammonium sorption can be negative with respect to plant growth, in the short term, if this effect is too great. This is because although ammonium can in principle be accessed by plants, ammonium sorbed to internal pores could be physically inaccessible to roots.

Some studies have also hypothesised effects of biochar on both mineralisation – the production of ammonium from the recycling of organic materials returned to soil (ammonification) – and nitrification. The mechanism from these effects is assumed chemical, i.e. a consequence of toxicity or change in background conditions such as soil solution pH. It could also itself be a feedback effect from decreased mineral nitrogen concentrations, since micro-organisms responsible for mineralisation require (as well as plants) nitrogen in order to proliferate. A few studies have proposed that biochar in some way protects nitrate, though the basis for such association is opaque (clearly there is no basis for such interaction on the basis of charge).

In addition to nitrogen, plants require an annual supply of phosphorus and potassium in order to grow. Phosphorus is largely immobile in soil due to its propensity, in solution, to rapidly form insoluble compounds – often with calcium and magnesium involved – and thus becoming firmly bound within the soil matrix. Plants are able to solubilise insoluble inorganic phosphate, however, for uptake alongside soluble phosphates by exudation of suitable compounds and enzymes from roots – in conjunction with suitable microbial association. Since phosphorus and potassium are both stable at pyrolysis temperatures biochar inherits the feedstock content, thermally mineralised and increased in concentration by the bulk partial elimination of carbon, hydrogen and oxygen. These elements are therefore potentially leachable, depending on the physical structure of the biochar.

The leaching of phosphorus and other mineral elements is constrained by the physical structure of biochar as well as its chemical composition. In the natural environment, the rapid weathering of charcoal from wildfire leads to expectation of similar changes in biochar after addition to soil. This is liable to affect the evolution of both direct and indirect interactions between plant roots, biochar and the soil solution in the months and years after addition.

Until now, the impact of biochar on soil nutrient cycling reported in published work has compared the status of the bulk soil after biochar addition, to control soils with no biochar applied. Although the visual association of plant roots and biochar and biochar and micro-organisms involved in nutrient recycling has been commented on and examined at micro-scale, comparison of the rhizosphere and bulk soil as a means to understand the diversity of biochar interactions has not been undertaken. Since changes in the physical form of biochar are likely to differ between materials derived from more and less woody feedstock, understanding the interaction of weathering effects and nutrient release could be important to devising deployment strategies in terms of frequency and rate of biochar addition.

In Objectives 4.2.1 and 4.2.2 we firstly sought an approach to assess and screen different biochar types for their effects on interaction of plants and nitrogen in the rhizosphere. Secondly, we sought an approach to screen for the impact of weathering on these effects and on the release of entrained phosphorus and potassium. Together, the application of these techniques to a subset of biochar samples could contribute to 'rules of thumb' for site-specific selection of biochar, together with application frequency and amount.

Methods

Screening for effects of biochar on plant–nitrogen interactions in the rhizosphere

Rhizoboxes are mesocosms that enable non-destructive monitoring of root growth and in this study, direct measurements of the soil-biochar-root interface. The purpose was to differentiate the concentrations of nitrate and ammonium in the rhizosphere and non-rhizosphere, and to determine the fate of nitrate in biochar-amended soil. Replicate rhizoboxes (n=6) were constructed from clear Perspex sheets screwed together to form an inner volume of 20 x 40 x 0.6 cm and packed with sieved sandy loam soil (pH 5.5; 0.55% carbon; 7.4 mg N kg⁻¹ soil). Fertiliser was added as follows: 18 mg N kg⁻¹ soil (KNO₃), 220 mg K kg⁻¹ (K₂SO₄) and 30 mg P kg⁻¹ (KH₂PO₄).

As a model biochar, 0.5–2.0 mm charcoal fines from mixed deciduous wood were used (Dalkeith Charcoal Co., Dalkeith, Scotland), mixed into the soil at 0.6 % or 1.9 % on a dry mass basis. This was

equivalent to an application of 20 and 60 t ha⁻¹ (BC20 or BC60 respectively). Control rhizoboxes received no biochar. Although rhizobox nitrogen contents ranged from 25.4 (control) to 53.4 (BC20) and 112.4 mg N kg⁻¹ (BC60), nitrogen in biochar is unavailable and potentially available nitrogen would have been close to ~25 mg N kg⁻¹ in all cases. One pre-germinated wheat seedling was added to each rhizobox and then incubated in a plant growth chamber for 29 days. An irrigation grid (3 mm diameter holes) on the back surface allowed water addition every three days. Silicone sealant around the box edges prevented water loss. The rhizoboxes were covered in aluminium foil to exclude light from the roots.

At harvest plant shoots were cut and dried. Roots were then carefully lifted and adhering soil and biochar particles subsequently shaken free designated “rhizosphere”. The soil and biochar remaining in the rhizobox was collected and designated “bulk soil”. Roots were washed over a fine mesh to remove tightly bound soil and biochar particle, imaged electronically using a flat-bed Scanner, then digitally analysed using commercial software (for root length and volume). Roots were then dried and as for the dried shoot material, milled and analysed for total C and N content. Rhizosphere and bulk soil were analysed for pH, ammonium and nitrate (plus nitrite, an intermediate). Samples of biochar particles (> 1 mm) were separately recovered from rhizosphere and bulk soil sub-samples and separately analysed for mineral N.

Differences between treatments were assessed by one-way ANOVA. Paired T-tests were used to compare rhizosphere and bulk soil. Differences were considered significant at $P \leq 0.05$; significant comparisons were tested using Fisher’s LSD. All analyses were conducted using Minitab v15.

Effect of biochar weathering on biochar interactions in the rhizosphere

Rather than using charcoal fines, 1.0–2.0 mm biochar from shredded elephant grass (*Miscanthus*) and chipped willow (*Salix*) – potentially available energy crop residues – were compared. *Miscanthus* biochar was produced in an auger-driven horizontal kiln at 700 °C by Pyreg Ltd, Germany; *Salix* biochar was produced in a batch reactor at 450 °C by J. Cook, University of Edinburgh, UK.

Each sample was adjusted to 60% water holding capacity. Half was artificially weathered by subjection to repeated rapid cycle freeze–thaw, 200 oscillations between 30 °C and -10 °C with 20 min hold. Fresh and weathered samples were analysed for pH (1:10 ratio), nitrogen, carbon (total, labile and stable), water holding capacity, bulk density, total P/K/Mg by modified dry ashing, and leachable nitrate, ammonium, P/K/Mg using a serial water extraction.

Rhizoboxes and soil used to develop the screening method, above, were used but the soil amended with mineral nitrogen at the equivalent of 40 kg N ha⁻¹. The four biochar types used (*Miscanthus* intact and weathered, M and wM; *Salix* intact and weathered, S and wS) were mixed into the soil at 0.3 % dry mass basis (equivalent to 10 t ha⁻¹). The control treatment had no biochar. Spring barley (rather than wheat) seedlings were planted. All parameters including duration were as above.

In addition to rhizosphere and bulk soil, the rhizosheath (soil strongly held to roots) was separately collected as sediment from washing with distilled water. Analysis of soil pH, ammonium and nitrate was as above. However, available soil phosphorus (acetic acid extractable) was additionally determined.

Biochar particles were then separately recovered from a portion of three randomly selected rhizosphere and bulk soils per treatment using fine forceps. All visible biochar particles (> 0.5 mm) were collected from a sub-sample, with any adhering soil particles removed. Biochar particles were analysed for ammonium and nitrate content.

Washed root samples were imaged as previously to analyse for root morphology (diameter classes) and root topology (branching pattern). The latter comprised the number of external links in the root system (root magnitude, μ) and the number of links in the longest path length (root altitude, a). Magnitude and altitude were used to determine the root system topological index, TI (a value close to 1.0 describes a “herringbone” root structure, a value close to 0.5 describes a “dichotomous” pattern). Leaves and roots were analysed chemically as previously.

Differences between treatments and within biochar types were tested using ANOVA.

Results

Screening for effects of biochar on plant–nitrogen interactions in the rhizosphere

Nitrate was statistically higher in the bulk soil compared to the rhizosphere (Fig. 1A&B). Bulk soil nitrate and ammonium (the latter lower than the former) were not affected by treatment.

Rhizosphere nitrate concentrations differed significantly between treatments (Fig.1B), especially between BC0 (0.6 ± 1.2 mg $\text{NO}_3\text{-N kg}^{-1}$ soil) and BC60 (4.5 ± 0.8 mg $\text{NO}_3\text{-N kg}^{-1}$ soil). Ammonium concentrations in the rhizosphere were below detection levels; low concentrations of NO_2^- were similar between treatments.

Nitrate was the dominant form of N form extracted from biochar particles. Although soil NO_3^- was lower in the rhizosphere, more NO_3^- ($\sim 60\%$) was extracted from biochar in the rhizosphere for both BC20 and BC60 than from biochar in bulk soil (160 mg N in the rhizosphere versus 98 mg N kg^{-1} in the bulk soil for BC60). Low concentrations of NH_4^+ were extracted from biochar in both BC20 and BC60 rhizosphere (~ 6.5 mg $\text{NH}_4\text{-N kg}^{-1}$ biochar) – and none from bulk soil biochar.

In order to calculate the total amount of mineral N held by the biochar, the proportion of rhizosphere and bulk soils and chars was estimated from remaining soil samples. The ratio of rhizosphere to bulk soil was 0.56:1, 0.57:1 and 0.61:1 for BC0, BC20 and BC60 respectively, with no significant difference between treatments. However, more mineral nitrogen (i.e. nitrate plus ammonium) was present in rhizosphere biochar for BC60 compared to BC20 (4.85 ± 0.33 mg compared to 1.06 ± 0.4 mg N biochar kg^{-1} soil), with a similar pattern for the bulk soil biochar (1.91 ± 0.34 versus 0.38 ± 0.08 mg N biochar kg^{-1} soil; see Fig. 1C). More mineral nitrogen was held in rhizosphere biochar in BC60 compared to the bulk biochar – but in BC20 rhizosphere biochar held only slightly more than its bulk soil counterpart.

Since NO_3^- is an anion, it is likely that NO_3^- was held in solution within biochar pores, rather than through cation exchange capacity, contrary to explanations proposed by previous studies.

There were significant treatment effects on pH in rhizosphere and bulk soils (Fig. 1D). For BC60, soil pH was lower in the rhizosphere (5.95) compared to bulk soil (6.03), with no significant difference between rhizosphere and bulk soil pH for BC0 (pH 5.56 and 5.49) or BC20 (pH 5.74 and 5.71).

The wheat plants achieved similar total biomass across the three treatments: 389 ± 20.8 , 366 ± 32.3 , 352 ± 27.5 mg dry wt in BC0, BC20 and BC60 respectively. Root lengths were also similar between treatments. Biochar addition had no effect on aboveground or root nitrogen content, or on total nitrogen content. However, root nitrogen uptake (inferred as mg plant N cm⁻¹ root length) was greater in the control than for the biochar treatments (Table 1).

There were no significant treatment effects on root traits (Table 1) such as root architecture (e.g. specific root length, SPL; root length density, RLD), or root morphology (root length to root volume and tissue density). Plant resource allocation belowground (root weight ratio) was not affected by biochar, but root length ratio was higher – for BC20 and BC60 roots were longer, increasing surface area and soil contact. This could provide a biotic explanation for the increased mineral nitrogen content in the rhizosphere of soils amended with biochar compared to the control, as there were correlations between RLR and root N uptake – and between root uptake of nitrogen and rhizosphere nitrate concentration.

Although biochar additions, especially at low rates (BC20), may increase root allocation without negative impacts to plant growth or quality, the potential for biochar to improve N use efficiency requires further attention as there was no correlation between root length ratio and mineral nitrogen in the rhizosphere.

Effect of biochar weathering on biochar interactions in the rhizosphere

Intact biochar from *Miscanthus* and *Salix* used in this work were similar in their carbon content, pH, bulk density and water holding capacity (Table 2). Both biochar types were highly stable and contained <1 % labile carbon. However, the biochar types did differ in their content of mineral nutrients – *Salix* biochar contained more phosphorus (1.56 ± 0.16 g P kg⁻¹) and Mg (1.83 ± 0.04 g Mg kg⁻¹), while *Miscanthus* biochar had higher potassium (18.33 ± 0.39 g K kg⁻¹) and Na (0.34 ± 0.006 g Na kg⁻¹). In terms of leachable nutrients, *Miscanthus* biochar released ~32% (0.27 ± 0.03 g P kg⁻¹) against only ~2.7% for *Salix* (0.04 ± 0.004 g P kg⁻¹) (see Table 2).

Simulated weathering resulted in no significant change in most measured properties of biochar from either *Miscanthus* or *Salix*. There were significant but small differences in leachable K, water holding capacity and bulk density. Scanning electron microscopy of biochar particles before and after weathering showed no evidence of notable structural change in pore structure.

Adding biochar to soil had an immediate effect on soil mineral nitrogen for most treatments, ranging from 34 ± 1.46 for no biochar to 61 ± 6.80 for M, 83 ± 17.19 for wM and 61 ± 16.2 for S. However, the weathered *Salix* biochar was similar to the control (33 ± 7.45 mg N kg⁻¹).

At harvest (after 28 d), the concentration and form of soil mineral N differed between bulk soil, rhizosphere and rhizosheath (Fig 2). Ammonium content was different between soil zones across all treatments, and was highest in the rhizosheath zone. However, there was no effect of biochar

addition or significant interaction. For nitrate, however, there was an effect on nitrate only in bulk soil, this being higher for the biochar treatments than the control. In the rhizosphere zone, nitrate was only detected in Miscanthus biochar treatments and there was no effect of weathering. No nitrate was detected in the rhizosheath zones of control or any of the biochar-amended soils.

Miscanthus biochar appeared to increase the pH of both bulk and rhizosphere by ~ 0.2 units, but this trend was also non-significant. Across treatments, bulk and rhizosphere zones were similar in pH, while pH was lowest in the rhizosheath soil zone. Adding fresh Miscanthus or Salix biochar to soil increased initial soil phosphorus contents by $\sim 0.6 \text{ mg P kg}^{-1}$ while adding weathered biochar made no change to soil P. After 28 d of growth, bulk soil P tended to be higher in treatments with fresh biochar. Overall, rhizosphere P content was generally lower compared to the bulk soil. However, there was no effect of biochar addition, nor significant interaction.

Shoot and root biomass were higher where biochar was added, and greatest for Miscanthus biochar (Table 3). Although there was no differences in shoot nitrogen content or root uptake, shoot phosphorus content, uptake and root content were higher than for biochar treatments (Table 3), especially for Miscanthus biochar. The increase in biomass was probably linked to soil P availability, since plant P uptake was correlated with root length.

Fresh or weathered Miscanthus biochar tended to display lower specific root length and root length ratio compared to the control, while plants growing in fresh or weathered Salix biochar displayed similar or sometimes higher root traits than plants growing without biochar (Table 3). However, there was no statistically significant effect. The topological data suggested that plants growing in weathered biochar amended soils had simpler, less branched root patterns compared to root systems in the fresh biochar soils – but differences in these traits were also non-significant.

Root diameters (Fig. 3) were categorised as main root, first order lateral roots and secondary fine roots. Root diameter distribution 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.

In the control treatment the rhizosphere was three times greater than the rhizosheath. The presence of Miscanthus biochar (M or wM) increased this ratio to four. Both the rhizosphere and rhizosheath zones were smallest for soils that received Salix biochar. Using a rhizosheath weight to root length ratio it was established that plants growing in soil amended with Miscanthus biochar had smaller rhizosheath compared to the control.

Results from hand-picking biochar revealed clear differences between bulk and rhizosphere (Fig. 5A). Biochar content was greater in the rhizosphere regardless of biochar type: 3.24 ± 0.06 and $3.79 \pm 0.16 \text{ mg biochar g}^{-1} \text{ soil}$ for Miscanthus and Salix respectively than in bulk soil (2.75 ± 0.09 and $3.08 \pm 0.11 \text{ mg biochar g}^{-1} \text{ soil}$). The difference was greater for Salix than Miscanthus, but weathering had no effect. There was an effect of biochar type on the ratio of biochar mass to root mass (Fig. 5B) with Salix biochar having the greater effect, but there was no effect of weathering on this parameter either.

Nitrate was the dominant form of nitrogen extracted from biochar, differently partitioned between soil zones according to biochar type (Fig. 6A and B). Approximately 6 and 4 $\mu\text{g N g}^{-1}$ soil was measured in Miscanthus biochar particles retrieved from bulk and rhizosphere samples respectively; weathering did not affect Miscanthus biochar-associated N contents ($\sim 3.5 \mu\text{g biochar-N g}^{-1}$ soil). Biochar-associated N content was an order of magnitude lower than the Miscanthus for soil amended with intact biochar from Salix.

When data on biochar-associated nitrogen were compared to the respective mineral nitrogen results by soil zone, Miscanthus biochar was found to account for one-quarter of total mineral N in the rhizosphere and one-half for the bulk soil (Fig. 6C). This trend was consistent between fresh and weathered Miscanthus biochar types. Although the concentration of mineral N was lower in the Salix biochar compared to Miscanthus (Fig. 6B), 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 at the end of the 28 d plant growth period.

Budgets were calculated for mineral nitrogen at the end of the experiment, presented graphically in Fig. 7. These capture the status of mineral N in each measured pool – shoot, biochar and soil in each zone – assuming no gaseous or leaching loss. Results are expressed as a proportion of rhizobox mineral N at the start of the experiment, after fertiliser addition (40 kg N ha^{-1}) and biochar amendment: control 126 kg N ha^{-1} , Miscanthus 221 kg N ha^{-1} , weathered Miscanthus 300 kg N ha^{-1} , Salix 222 kg N ha^{-1} and weathered Salix 120 kg N ha^{-1} (the small amount of N introduced in the biochar amendments was excluded on account of its recalcitrance).

The budget shows that biochar maintained higher mineral nitrogen concentrations in bulk and rhizosphere soil zones compared to the control, with differential partitioning of N between soil and biochar greater for Miscanthus-derived material than the Salix biochar. Importantly, this retention of nitrogen did not affect plant nitrogen uptake, which was similar across treatments (see also Table 3). The proportion of mineral nitrogen that was biochar-associated varied with biochar type: the Miscanthus biochar retained more nitrogen than the Salix biochar, and this was decreased by weathering. The presence of Miscanthus biochar increased the nitrogen localisation into the rhizosphere but weathering appeared to lower this effect. In the Salix biochar treatment, there was no differential partitioning between biochar and soil in either bulk or rhizosphere zones. The remaining N has been termed ‘immobilised N’ as microbial biomass N was not independently measured and is the missing pool from the data set. Root N content and rhizosphere biochar-N were also not quantified but are likely to form smaller pools in comparison to the microbial pool. The higher immobilisation in the control and Salix biochar treatments suggests that the microbial community in these treatments may have been nutrient limited compared to the Miscanthus biochar treatments. This treatment will have received additional leachable biochar-P, which may have reduced immobilisation.

Interpretation

Drawing on the results from the first part of this work, we propose that a direct physical mechanism such as mass flow is responsible for rhizosphere nitrate localisation around biochar. The gradient in nitrate concentrations between bulk and rhizosphere soil was much greater for BC0 compared to

BC20 and BC60, suggesting that biochar may interface the flow of nitrate from soil to plant. Correlations between roots and soil nitrogen suggest that a biotic influence is also relevant, plants in BC0 showing higher N uptake rate than plants in biochar-amended soils (Table 1). This means that nitrate localisation around roots may moderate the urgency of uptake of N by plants.

Evidence from other studies indicates that biochar can decrease leaching and nitrous oxide emissions. Localisation of nitrate within rhizosphere could protect nitrogen against loss where plant uptake is lower.

This initial work established the potential for the rhizobox approach to assist in the appropriate selection of biochar or its application rate. As site-specific data on the agronomic impacts of biochar becomes more abundant, the need to tailor application rates to optimise between carbon storage and agronomy is becoming clearer.

Building on this demonstration of a screening tool, the localisation of nitrogen around biochar was tested with alternate biochar types, examining whether nitrogen retention was linked to biochar being preferentially situated within the rhizosphere.

Biochar was used at lower concentration (equating to 10 t ha^{-1} , rather than 20 and 60 t ha^{-1}), but nitrogen retention and localisation remained apparent. Additionally, biochar was found to be preferentially located within the rhizosphere – so biochar partitioning can occur. Since the localisation of mineral N in biochar and the preferential partitioning of biochar into the rhizosphere were sensitive to biochar type, screening appears to be important.

Manipulating biochar is a useful tool for understanding the durability of biochar properties, and could be developed to predict longer-term biochar function in soil. However, in this work the manipulation of *Miscanthus* and *Salix* biochar samples using an artificial weathering approach based on freeze–thaw did not much change biochar properties. The simulated weathering process applied may have altered biochar structure, although to a minor degree, such as collapsed or clogging of pores affecting accessibility to extractants, water holding capacity, bulk density and nutrient release by leaching.

Although the intrinsic differences between *Miscanthus* and *Salix* biochar types were greater than any change brought about by artificial weathering, there was a difference between fresh and manipulated biochar in relation to biochar–root interactions. At a micro-scale the measured impact on water holding capacity and bulk density could explain alterations relevant to root hairs and pore-water dynamics not captured by the methods reported in Table 2. Overall, subtle effects of weathering measured in this study suggest that working with manipulated biochar could be useful in predicting the long-term function of biochar in soil, especially if an extended or stronger weathering procedure could be defined. Ideally this would enable biological as well as chemical and physical effects to be assessed separately and in combination.

Comparing total and leachable nutrients, *Miscanthus* biochar had greater potential to supply mineral nutrients than did *Salix*. Although the 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. The effects of biochar

nutrient quality were apparent in the N budget (Fig. 7) and in the positive shoot P uptake and root responses resulting from addition of *Miscanthus* biochar. However, other interactions such as physical, chemical or microbial immobilisation occur once biochar is incorporated into the soil (see Fig.1).

Although biochar does not provide a direct source of N, our data shows that soil-recovered biochar retains mineral nitrogen, mainly in the form of $\text{NO}_3\text{-N}$. It appears that biochar impacts the retention of biotic nitrogen release in soil, with soil extractable nitrogen including a discrete biochar-nitrogen pool that can be of potentially important magnitude – up to one-quarter or one-half of soil mineral N, depending on soil zone (bulk soil or rhizosphere) and biochar type (more evident for *Miscanthus* biochar compared to *Salix*-derived).

At the end of the plant growth period in this work, mineral nitrogen remaining in soil was the balance between retention and localisation, root uptake and immobilisation. Nitrate was the dominant form of nitrogen in biochar particles in soil, in contrast to the findings of other studies that generally infer low association of nitrate and biochar, from analysis only of bulk soil. The difference between the retention of nitrogen by *Miscanthus* and *Salix* biochar also reflects differences between these biochar types in feedstock type and pyrolysis temperatures, which are known to affect biochar structure and porosity (which impact biochar retention) and labile C content (which would determine microbial activity).

Distinguishing bulk soil from those soil zones directly affected by the plant (rhizosphere and rhizosheath) enabled the impacts of biochar most pertinent to plant growth to be established. In field based research work, increased nitrate and decreased ammonium concentrations have been reported for various cereal and crop plants after biochar addition. The results from work in this project, however, indicate that the retention or localisation of nitrate within biochar particles had no negative effect on plant growth and nitrogen uptake (see Table 3). Soil nitrate retention where biochar was present could have been due to nitrate concentrations exceeding the demand of the plant. or biochar particles adsorbing labile carbon exuded by roots, stimulating nitrification around microsites within biochar (converting ammonium to nitrate).

Quantification of biochar particles within different soil zones demonstrated that biochar partitioning can occur: more biochar was located in the rhizosphere compared to bulk soil. Since biochar was not mobile in the rhizoboxes used in the study, it was conclusively shown – for the first time – that plant roots proliferate around biochar particles. As the size of the rhizosphere and development of the rhizosheath indicate the foraging preferences of roots, it also seems that roots seek out biochar in soil.

In common with the initial rhizobox study, there was no significant biochar effect of biochar on root traits, even though *Miscanthus* biochar led to an increase in root biomass. Such detailed assessment of root responses to biochar and effects on root function are lacking in the literature.

Root rhizosheath formation is common in sandy soils and is directly related to root hair length and density: root hairs enmesh sand particles which are held together through root mucilage and soil moisture interactions. While rhizosheaths are important for root acquisition of soil water,

development of the rhizosphere also improves soil aggregation, soil structure, and increases C stabilisation. In addition, root hair development is the key mechanism employed by plants to facilitate P uptake, as increased root hair length and density are also directly related to improved P uptake under P deficient conditions (Richardson et al., 2009).

Our measurements of rhizosphere development suggest that biochar–root interactions are mediated mainly at the micro-scale of root hairs and biochar pores (Fig. 1). Root systems growing in the control treatment had large rhizospheres compared to the biochar treatments; large rhizospheres are associated with longer and denser root hairs shown elsewhere to facilitate water and phosphorus uptake. It is likely that in the absence of biochar-associated phosphorus, a large rhizosphere developed. Where biochar was added biochar-associated P – more for Miscanthus biochar – probably resulted in the smaller rhizospheres. Smaller rhizospheres in the biochar treatments could also be a response to potential increased water retention within biochar pores that could improve water uptake by roots. More biochar was located within the rhizosphere than bulk soil (Fig. 5), indicating a positive biochar–root interaction. Slightly more biochar was retrieved from the Salix treatment rhizosphere (Fig. 5b), despite the low capacity of Salix biochar to provide leachable phosphorus. This suggests that differences in pore size and structure between Salix and Miscanthus biochar types may regulate root responses to biochar.

Uptake of mineral nitrogen by root hairs could have taken place as a consequence of nitrate retention in biochar, being largely a product of diffusion and mass flow, especially for nitrate. Higher concentrations of biochar in the rhizosphere probably made access of nitrate easier. Root length was negatively correlated with shoot nitrogen content, confirming less investment in root production to acquire nitrate.

Conclusions

This work establishes that biochar affects soil–plant relationships with respect to nitrogen, also to provide a direct source of leachable/accessible nutrients for plant roots, at rates of application as low as 10 t ha⁻¹ biochar.

It defines some approaches by which rules of thumb might be developed for selection of biochar to match site specific requirements, where leaching and nitrous oxide emission as well as crop productivity and nutrient use efficiency are relevant considerations.

Understanding the relationship between pore-structure, pore size and water flow will be important in defining the mechanisms involved in biochar nutrient retention and reduced leaching further.

Further work is required to determine whether biochar nutrient retention within the rhizosphere or bulk soil occurs for other soil nutrients.

Findings and biochar selection based on this work will need to be validated in the field, to demonstrate that the screened mechanisms are important to crop plants under field conditions.

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