

Biochar addition to soil changed microbial community structure and decreased microbial biomass carbon and net inorganic nitrogen mineralised

Daniel N. Dempster^A, Deirdre B. Gleeson^A, Zakaria M. Solaiman^A, David L. Jones^B, Daniel V. Murphy^A

^ASchool of Earth and Environment (M087), Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley, WA, 6009. Email: dempsd01@student.uwa.edu.au

^BSchool of the Environment and Natural Resources, University of Wales, Bangor, Gwynedd LL57 2UW, UK

Abstract

Biochar is a recalcitrant carbon rich soil amendment proposed to improve soil fertility. We investigated changes in soil microbial biomass, community structure and function and net inorganic nitrogen changed resulting from its addition. We homogeneously incorporated biochar at rates of 0, 5, and 25 t/ha into a coarse textured sand in a glasshouse trial. Three nitrogen treatments were added: organic nitrogen (a pelleted pig manure and wheat straw compost), inorganic nitrogen (33 mg kg⁻¹ of N as NH₄NO₃ at 3 and 6 weeks), and a control treatment. Wheat (*Triticum aestivum* cv. Calingiri) was grown for 70 days, after which samples were taken. Microbial biomass carbon (MBC) decreased with biochar addition (P=0.05). Microbial carbon to nitrogen ratio and microbial community structure changed significantly with biochar addition at 25 t/ha but not at 5 t/ha. The decrease in microbial C: N ratio (from 8:1 to 5:1) suggests a shift to a more bacteria dominant community. Net nitrogen mineralisation decreased from an average of 11 mg N/kg dry soil after 10 weeks of incubation without biochar to 1 mg N/kg dry soil with addition at 25 t/ha. Sorption of nitrogen to biochar at these rates could not explain the result.

Key Words

Biochar, microbial biomass carbon, net inorganic N, microbial community structure, nitrification

Introduction

Biochar is the remains of pyrolysis of plant or animal based organic matter, where pyrolysis is the process of heat induced (> 250°C) anaerobic decomposition of organic matter (Antal and Grønli, 2003). Biochar can be stable in soil for thousands of years and is being proposed as an amendment to sequester carbon in agricultural soils. The implications of biochar additions on the soil microbial community are less clear. Investigation of this interaction has predominantly shown that the soil microbial biomass and/or microbial activity has increased with biochar additions (e.g. Steiner *et al.*, 2008; Kolb *et al.*, 2009). It has been hypothesised that biochar can provide a microbial refuge due to its porous nature (Peitikainen *et al.*, 2000).

The size of the microbial community can be linked to nitrogen mineralisation within the soil (Zaman *et al.*, 1999). As a large portion of crop nitrogen is derived from biological processes, changes in microbial processes derived from biochar addition to soil must be both investigated and documented. Biochar additions can enhance net nitrification in pine forest ecosystems (DeLuca *et al.*, 2006); such changes have yet to be seen in agricultural soils. This research examined the interaction between the soil microbial community and nitrogen mineralisation, as induced by biochar addition to soil. We hypothesised that the addition of biochar to soil would, via microbial habitat provision (Peitikainen *et al.*, 2000) induce an increased microbial biomass. We also hypothesised that nitrogen mineralisation would increase, due to the increased microbial biomass and its intimate link to enzyme production (Zaman *et al.*, 1999). To test this hypothesis a pot trial was designed in which biochar was added to a sandy soil, due to its low surface area and lack of microbial refuges, in which wheat was grown.

Materials and Methods

Setup and Experimental Design

Soil (0–10 cm) was collected from a site 20 km North-West of Moora, Western Australia. Texture and basic soil chemical properties and were assessed. The experimental design consisted of two factors: biochar (3 levels, fixed) and nitrogen treatment (3 levels, fixed) with four replicates in a randomised block. Biochar was added to soil at rates of 0, 5 and 25 t/ha equivalent (volumetric basis; 0.45 % and 2.27 % w/w respectively) and incorporated homogeneously into 2.2 kg of soil into sealed pots.

Pots were also treated with either organic nitrogen, as 500 kg ha⁻¹ equivalent of a wheat straw and pig manure compost (Custom composts), inorganic nitrogen, 33.3 mg kg⁻¹ of N as NH₄NO₃ at 3 and 6 weeks or

no added nitrogen, the control treatment. All pots were watered to 50 % moisture holding capacity and three wheat plants (*Triticum aestivum* cv. Calingiri) grown per pot. Soil and plants were collected for analysis ten weeks later.

Sample Analysis

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined using the fumigation extraction method (Vance *et al.*, 1987) and the ninhydrin method (Joergensen and Brookes, 1990) respectively. Community level physiological profiles (CLPP) were measured by the method of Degens and Harris (1997).

DNA extraction, PCR and Terminal Restriction Fragment Length Polymorphism (T-RFLP)

DNA was extracted from soil as in Griffiths *et al.* (2000). PCR amplification of the *amoA* gene was based on the method of Horz *et al.* (2000), using the primer sets amo-1F and amo-2R (Rotthauwe *et al.*, 1997). Approximately 100 ng of purified PCR product was used in a restriction digest with the endonuclease enzyme HaeIII (New England Biolabs Inc.). Terminal restriction fragment lengths were determined by electrophoresis and analysis of fragment profiles was carried out using Genemapper.

Net Inorganic Nitrogen

Soil was adjusted to 40 percent water holding capacity and incubated at 15°C. Samples were taken weekly for six weeks and at ten weeks and extracted by shaking 20 g (dry weight equivalent) soil with 60 mL of 0.5 M K₂SO₄ for one hour in 120 mL vials. The extract was filtered and analysed colourimetrically for NH₄⁺ using the salicylate-nitroprusside method (Searle 1984) and NO₃⁻ concentration using the hydrazinium reduction method (Kempers and Luft, 1988) on an automated flow injection Skalar Auto-analyser (Skalar San plus).

Statistical Analysis

MBC, MBN, microbial C:N ratio, microbial respiration, and net nitrogen mineralisation data was analysed by performing a series of general analyses of variance in GENSTAT 10th Edition (Lawes Agricultural Trust 2007). Multiple mean comparisons were done using Duncans multiple range test; significance was considered at $P \leq 0.05$. Multivariate statistical analyses were performed on standardised, (Log (X+1)) transformed CLPP data and standardised, untransformed T-RFLP profiles using Primer 6 (Primer-E Ltd, UK) using permutational multivariate analysis of variance (PERMANOVA).

Nitrogen Sorption

The factors in this incubation were soil type (four levels, fixed), nitrogen solution (four levels, fixed) and nitrogen solution concentration (10 levels, fixed). 10 g of either soil, biochar, or soil biochar mixes (5 or 25 t/ha equivalent) were mixed with 100 mL of nitrogen solution (either Leucine, (NH₄)₂SO₄, KNO₃, NH₄NO₃) in a 120 mL vial. The nitrogen concentrations used were 0, 0.5, 1, 1.67, 2.5, 5, 10, 25, 50 and 100 mg N/l for (NH₄)₂SO₄ and KNO₃ and double for NH₄NO₃. This mixture was shaken for 24 h, centrifuged for 5 minutes at 4000 rev/min and filtered (Whatman N^o 42). This was then repeated, except after shaking for 24 h, K₂SO₄ was added to 0.5 M, and the solution was shaken for another hour, after which the solution was centrifuged, filtered and stored as stated previously. Inorganic and organic nitrogen extractions were measured using the automated flow injection, as previous. Significant differences were considered when the original solution concentration was greater than the 95 % confidence interval of the solution concentrations after shaking.

Results

The soil used contained 95 % sand, 1.5 % silt and 3.5 % clay. The pH of biochar was 7.43, but its addition did not significantly increase soil pH from 4.76. Pore space potentially habitable by microbes, deemed as the proportion greater than 0.60 µm, in the biochar was 4.42 m²/g, six times greater than the soil (0.74 m²/g) but this pore size equated to 0.76 and 0.82 m²/g at the added rates.

Microbial population analysis

Microbial biomass carbon declined (from 192 mg/kg) with biochar addition. When added at 5 t/ha (158 mg/kg) the difference was not significant, as it was when added at 25 t/ha (128 mg/kg) (Figure 1). There was no interaction between the rate of added biochar and the nitrogen treatment. The treatments did not induce any differences in MBN. The initial microbial biomass C: N ratio was 8: 1. This was not different from biochar addition at 5 t/ha, however the ratio declined to 5:1 with addition at 25 t/ha (Figure 1). The CLPP of the 25 t/ha biochar treatment was significantly different from the treatments without biochar or where added

at 5 t/ha ($P < 0.01$). The nitrogen treatment had no effect of CLPP and there was no interaction between the factors. T-RFLP analysis showed that overall there was a significant effect of biochar and there was interaction between the two factors. Without a nitrogen treatment, there was no effect of biochar on the nitrifying bacterial community. However in the presence of compost and inorganic nitrogen, the nitrifying bacterial community changed with rates of added biochar.

Net Inorganic Nitrogen

Inorganic nitrogen accumulation significantly declined with increased biochar application rate (Figure 2). After 10 weeks of incubation the inorganic N concentration was an average of 11 mg/kg without biochar, 7 mg/kg where added at 5 t/ha and 1 mg/kg where added at 25 t/ha. ANOVA showed that at the end of the incubation, there was no significant effect of nitrogen treatment.

Nitrogen Sorption

At the rates of biochar applied to soil recovery of both ammonium and nitrate was *ca.* 100% indicating no significant sorption of either form of inorganic N. However in pure biochar, 0.5M K_2SO_4 could only extract around 65 % of the ammonium up to a concentration of 50 mg kg^{-1} , 80 % from 50 mg/kg to 250 mg/kg, and most ammonium at greater concentrations (Figure 3). However, with 0.5 M K_2SO_4 it was possible to recover only 5 % nitrate (i.e. 95 % sorption) up to 120 mg/kg. Above this rate nitrate was recovered (approximately 50 %) indicating saturation of the exchange surface.

Conclusions

Contrary to our expectations and previous studies (eg. Kolb *et al.*, 2009), the microbial biomass carbon decreased with added biochar. The altered microbial C: N ratio, combined with altered CLPP profiles, suggest a shift to a more bacterial dominant community when biochar was added at 25 t/ha. The addition of biochar to soil also decreased the amount of net inorganic nitrogen (Figure 2). This could not be attributed to immobilisation, as MBC decreased with biochar addition and MBN did not change. This change could also not be due to lesser mineralisation, as C and N mineralisation are intimately linked (Mazoni *et al.*, 2008) and microbial respiration did not change with addition at 25 t/ha. The bacterial nitrifying community did not change with biochar addition alone. Sorption may provide some explanation as large amounts of inorganic N (especially nitrate; Figure 3), could not be recovered when mixed with pure biochar. However, there was almost no sorption when biochar was added to soil, probably partially due to surface area differences. There must also be other mechanisms inducing the decreased inorganic N with biochar addition.

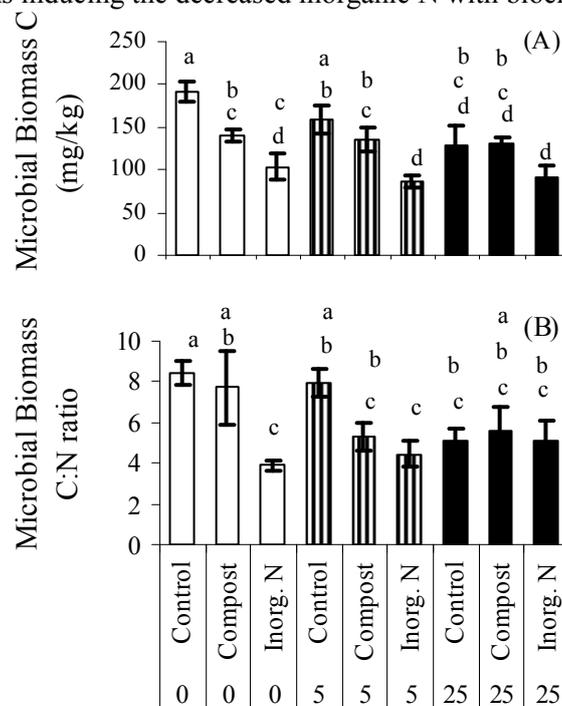


Figure 1: Microbial biomass carbon (A) and Microbial biomass C: N ratio (B) results for each nitrogen treatment at three rates of added biochar (0, 5 and 25 t/ha). Error bars represent standard errors (n=4) and letters, significant differences.

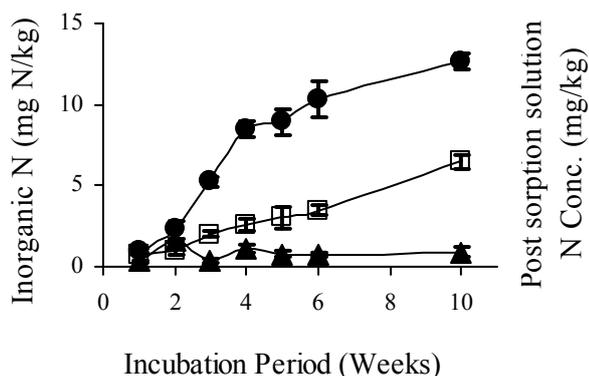


Figure 2. Total inorganic nitrogen extracted from the a representative nitrogen treatments for biochar added at 0 (●), 5 t ha⁻¹ (□) and 25 t ha⁻¹ (▲). Error bars represent standard errors (n=4).

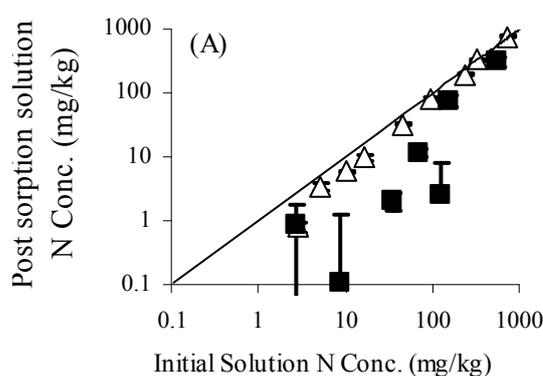


Figure 3. Sorption of Ammonium (Δ) and Nitrate (■) to biochar. Error bars represent standard errors (n=3).

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