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Comparative analysis of the microbial communities in agricultural soil amended with enhanced biochars or traditional fertilisers

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ABSTRACT

Biochar can have a positive effect on agricultural soils and plant yields. The underlying mechanisms that deliver beneficial outcomes are still poorly understood. Soils contain complex communities of hundreds or thousands of distinct microorganisms, and it has been shown that biochar can have an impact on their composition and function. Here we analyse the microbial communities in a controlled field trial that compared the effect of enhanced biochars (EBs) against a farmer practice (FP) of traditional fertilisation (urea, superphosphate and potash) on sweet corn yield. During sequential crop cycles (barley and sweet corn) two types of EBs were applied at low and high levels (total of 1.1 and 5.44 t ha^{-1}, respectively). Samples were taken at the end of a second crop cycle and over 50,000 16S ribosomal RNA (16S rRNA) tag sequences were generated per sample to characterise microbial communities. Despite the lower amounts of nutrients provided by EBs, their amendment to soil produced similar crop yields to the FP. In addition, significant differences in microbial community composition were observed between the high EB and FP treatments. This was driven by differences in the relative abundances of only a few community members. Community level differences were also correlated with a higher soil pH associated with EB laden soil. Network analysis showed that the low EB application had more correlation patterns (co-occurrences and exclusions) between microbial taxa, and highlighted the importance of associations between members of the phyla Acidobacteria and Verrucomicrobia in the biochar environment. Overall, a large number of microorganisms appear to be influenced by EB amendment compared with fertiliser use leading to a complex re-wiring of community composition and associations.

1. Introduction

Biochar is carbon-rich organic matter derived from the thermal processing of biomass in an air-starved environment. Despite being a by-product of biomass conversion to biofuels, biochar has only recently received increasing attention as a form of carbon sequestration and a beneficial soil amendment in modern agricultural practices (Lehmann and Joseph, 2009). Historical use of black carbon as a soil amendment, through slash-and-burn techniques, has been observed globally by indigenous cultures in Australia, Africa, South America and Asia, highlighting the beneficial effects of pyrogenic carbon amendment in soils (Joseph et al., 2013).

The increased fertility of biochar-laden soil has generally been associated with physical and chemical changes in soil characteristics, such as nutrient retention (Laird et al., 2010), higher water holding capacity, higher cation exchange capacity (CEC) and increases in pH for acidic soils (O'Neill et al., 2009; Anderson et al., 2011; Lehmann et al., 2011). However, changes to microbial communities in response to biochar, which are associated with increased soil productivity, nutrient turnover and nutrient utilisation, offer additional explanations for the agronomic benefits (Anderson et al., 2011; Kolton et al., 2011). This is supported by the example that the carbon-rich, highly fertile "Amazonian dark soils" (Arthrosols) possess distinct communities of microorganisms when compared with adjacent carbon-poor soil, and show increases in microbial diversity and microbial biomass (O'Neill et al., 2009; Grossman et al., 2010). This suggests that biochar addition increases the niche space for soil microorganisms.

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How biochar changes the microbial communities in contemporary agricultural sites and practices is still poorly understood, with many studies using pot trials as proxies for field applications (Anderson et al., 2011; Kolton et al., 2011). Field-scale investigations of microbial communities in soils influenced by biochar are limited to historical (O'Neill et al., 2009; Grossman et al., 2010) or fire-impacted sites (Khodadad et al., 2011). Furthermore, how biochar influences microbial communities compared with traditional (NPK) fertiliser addition is unknown. If the addition of biochar changes microbial communities and their functioning to mediate an efficient use of nutrients or to alter biogeochemical processes within soil, then this may result in similar crop yields as traditional fertiliser practices. Such a scenario would then be reflected in the changes of the abundance and the interactions between microorganisms under biochar amendment.

In this study, we investigate soil microbial communities in an agricultural field trial that examines the effect of enhanced biochars (EB), consisting of biochar supplemented with various minerals (a biochar-mineral complex), on sweet corn yields. We compared a farmer practice (FP) of NPK fertiliser use against the addition of the EBs at both low (1.1 t ha^{-1}) and high (5.44 t ha^{-1}) levels applied over two crop cycles 7 months apart. Soil microbial communities were analysed using high-throughput sequencing (Illumina HiSeq 2000 platform) of the 16S rRNA gene. We identified a number of key microbial taxa that are different in relative abundance between treatment and used network analysis to understand the associations between microbial taxa in biochar compared with fertiliser amendment.

2. Methods

2.1. Materials and field experiments

Biochar (Anthroterra Pty Ltd, Somersby, Australia) was produced from jarrah wood (Simcoa Pty Ltd, Bunbury, Western Australia) in a vertical retort with a residence time of approximately 12 h and a maximum temperature of 600 °C. This biochar was activated with phosphoric acid and mixed with high iron bearing kaolinitic clay (30%, w/w), chicken manure (30%, w/w), rock phosphate and various other minerals, including basalt dust illmenite and dolomite (the specific recipe is proprietary). This mixture was torrefied at 220 °C for 3 h to produce two enhanced biochar (EB) products, EB7 and EB17, whose properties are given in Table 1. Biochar was tested for total P, water-soluble P, citrate-insoluble P, and available P (water-soluble + citrate-soluble) according to AOAC Official Methods 977.01 and 963.03 (AOAC International, 2000).

Agricultural field trials with EB were conducted at Department of Primary Industries' testing site in Wollongbar, NSW, Australia (28°50′ S, 153°25′ E). This site is characterised by a highly permeable red Ferrosol derived from tertiary basalt (Nicholls et al., 1953). The field site is located in a subtropical zone with predominant rainfall in summer. Previous to the field becoming a trial site, it had been managed as a dairy pasture from ca. 1905 through to 2005, when management reverted to a much lower input grazing pasture. The field site was prepared by mowing existing kikuyu pasture and removing most of the above ground biomass. The site was sprayed with glyphosate and was rotary hoed to 100 mm two weeks later.

Prior to the sweet corn trial described here, a winter barley crop was grown at the same field site investigating the use of EB7. Carry-over effects as a result of biochar residence time were therefore taken into consideration and are presented here. Prior to sowing a winter barley crop in May 2010, EB7 was applied at either a low (1 t ha^{-1}) or high (5 t ha^{-1}) application level. The site was again rotary hoed to incorporate the amendments into the 0–100 mm soil layer, while a farmer practice (FP) of a nitrogen fertiliser application (214 kg ha^{-1} of Norco Forage Starter) was surface applied following seeding to plots not containing the EB. This experimental design was based around different management practices and therefore plots consisting of no amendments were not included. The Ferrosol of the field site also has very low availability of P and would not support significant crop growth (Slavich et al., 2013). Barley was harvested 5 months later and the field site was sprayed and rotary hoed.

Table 1
Chemical properties of the enhanced biochars used during the barley (EB7) and sweet corn field trials (EB17). n.d = no data.

	EB7	EB17
Carbon (%)	62	31.8
Hydrogen (%)	2	1.06
Nitrogen (%)	0.62	1.24
Total sulphur (%)	0.03	0.25
Oxygen (%)	15.57	10
Moisture (%)	1	2.9
Ash content (%)	7.8	54
Volatile matter (%)	16.2	17.6
Fixed carbon (%)	75	28.4
$H:C_{org}$	0.03	0.04
$pH_{(H2O)}$	8.56	n.d
$pH_{(CaCl2)}$	6.8	6.6
Electrical conductivity (mS cm^{-1})	1.67	8.1
Total P (mg kg^{-1})	29,000	31,000
Water soluble P (mg kg^{-1})	2100	2200
Citrate insoluble P (mg kg^{-1})	19,000	2400
Citrate soluble P (mg kg^{-1})	7300	26,000
Available P (mg kg^{-1})	9400	29,000
NH_4 (mg kg^{-1})	660	45
NO_3 (mg kg^{-1})	0.55	0
Acid neutralising capacity (% $CaCO_3$)	14	12

Two months after the barley harvest, the site was prepared for the sweet corn crop *Zea mays* var H5 used in this study. Prior to sowing, 0.1 t ha^{-1} EB17 was applied into the low application plots, while 0.44 t ha^{-1} was applied into the high application plots. Therefore a total of 1.1 and 5.44 t ha^{-1} existed in the low and high plots, respectively, after the second application of biochar. The FP treatment applied to the sweet corn crop consisted of 400 kg ha^{-1} urea (184 kg ha^{-1} N), 300 kg ha^{-1} of single superphosphate (78 kg ha^{-1} P) and 140 kg ha^{-1} of potash (70 kg ha^{-1} K). Treatments were added to plots in triplicate which were positioned within three arrays. Each array contained one of each treatment (i.e. a randomised complete block design, RCBD).

At sweet corn harvest (3 months after sowing), the mass of corn cobs, the number of corn cobs and the dry biomass of corn stover was measured and converted to the yield of each of these variables per ha. Soil samples (0–100 mm) were also collected within each plot. Three replicate soil subsamples without roots were collected randomly but ensuring a distance of at least 5 m apart between collections. For microbial community analysis, the three subsamples were analysed separately (this allowed for an extra within plot 'subsampling' factor in the RCBD). For chemical analysis of the soil, the three subsamples were combined for one analysis per plot. For the soil samples, measurements of pH, electrical conductivity (EC), extractable phosphorus (Bray or Colwell extraction methods), plant available ammonium and nitrate, total carbon and nitrogen were conducted in an ISO17025 accredited laboratory using methods described in Van Zwieten et al. (2010). Microbial biomass carbon and microbial activity were analysed according to Bell et al. (2006).

2.2. DNA extraction and 16S rRNA gene sequencing of soil microbial communities

Microbial communities in soil were analysed as part of the Earth Microbiome Project (EMP, Gilbert et al.,