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Wood biochar increases nitrogen retention in field settings mainly through abiotic processes

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ABSTRACT

Nitrogen (N) is an essential element associated with crop yield and its availability is largely controlled by microbially-mediated processes. The abundance of microbial functional genes (MFG) involved in N transformations can be influenced by agricultural practices and soil amendments. Biochar may alter microbial functional gene abundances through changing soil properties, thereby affecting N cycling and its availability to crops. The objective of this study was to assess the effects of wood biochar application on N retention and MFG under field settings. This was achieved by characterising soil labile N and their stable isotope compositions and by quantifying the gene abundance of *nifH* (nitrogen fixation), *narG* (nitrate reduction), *nirS*, *nirK* (nitrite reduction), *nosZ* (nitrous oxide reduction), and bacterial and archeal *amoA* (ammonia oxidation). A wood-based biochar was applied to a macadamia orchard soil at rates of 10 t ha^{-1} (B10) and 30 t ha^{-1} (B30). The soil was sampled after 6 and 12 months. The abundance of *narG* in both B10 and B30 was lower than that of control at both sampling months. Canonical Correspondence Analysis showed that soil variables (including dissolved organic C, NO_3^-–N and NH_4^+–N) and sampling time influenced MFG, but biochar did not directly impact on MFG. Twelve months after biochar application, NH_4^+–N concentrations had significantly decreased in both B10 (4.74 μg g^{-1}) and B30 (5.49 μg g^{-1}) compared to C10 (13.9 μg g^{-1}) and C30 (17.9 μg g^{-1}), whereas NO_3^-–N concentrations increased significantly in B30 (24.7 μg g^{-1}) compared to B10 (12.7 μg g^{-1}) and control plots (6.18 μg g^{-1} and 7.97 μg g^{-1} in C10 and C30 respectively). At month 12, significant $\delta^{15}N$ of NO_3^-–N depletion observed in B30 may have been caused by a marked increase in NO_3^-–N availability and retention in those plots. Hence, it is probable that the N retention in high rate biochar plots was mediated primarily by abiotic factors.

1. Introduction

Reliance on inorganic fertiliser is increasing globally to meet the needs of a growing population for food production (Lal, 2004; Bouwman et al., 2013). However, inorganic nitrogen (N) inputs do not always ensure high yields as plant-available N can be lowered through leaching, sorption and volatilisation (Jaynes et al., 2001). Furthermore, the movement of N into ground water and the atmosphere can have negative environmental impacts (Thorburn et al., 2011). Therefore, N-use efficiency needs to be maximised in cropping systems (Bramley and Roth, 2002; Manlay et al., 2007). Previous studies have shown that adding organic matter residues to the soil improves soil N retention (Blumfield and Xu, 2003; Bai et al., 2014; Reverchon et al., 2015) and provides labile carbon (C) sources to the soil microorganisms involved in N transformations (Steiner et al., 2008). Biochar is a C-rich product of the pyrolysis of different feedstocks such as crop residues, wood chips, poultry litter or manure (Lehmann and Joseph, 2009; Bai et al., 2015; Xu et al., 2015) and is used as a soil amendment to increase soil

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quality (Lehmann, 2007). Biochar affects N cycling through different mechanisms including sorption of NO_3^-, NH_3, NH_4^+ and organic-N as well as through changes in microbial processes and activities (Van Zwieten et al., 2010a, 2010b; Cayuela et al., 2014; Van Zwieten et al., 2014). However, these processes may reduce N availability to plants (Deenik et al., 2010). Biochar also alters cation and anion exchange capacity in the soil, which further influences N retention (Clough et al., 2013; Slavich et al., 2013). Feedstock, production temperature, residence time at maximum temperature and post biochar treatments may influence the retention of NO_3^-–N and NH_4^+–N (Clough and Condron, 2010; Mukherjee et al., 2011; Ippolito et al., 2012; Reverchon et al., 2014). However, recent evidence has shown that the N adsorbed by biochar can eventually become available to plants (Taghizadeh-Toosi et al., 2012a, 2012b).

The mechanisms through which biochar influences N availability and thus plant productivity remain largely unclear, although they seem to be principally mediated by microbial processes (Güereña et al., 2013; Anderson et al., 2014). The effects of biochar on microbially-mediated processes such as nitrification, denitrification and N fixation have been previously investigated (Rondon et al., 2007; Spokas and Reicosky, 2009; Van Zwieten et al., 2014, 2015). However, the influence of biochar on microbial functional genes (MFG) involved in N-cycling is still poorly understood. Recently, Ducey et al. (2013) and Harter et al. (2014) reported that biochar enhanced the abundance of MFG involved in N fixation, nitrification and denitrification, while Van Zwieten et al. (2014) suggested biochar increased the abundance of *nosZ* and hence reduced the emissions of N_2O, most likely through an increase in soil pH by 1–1.3 units. However, not all biochars increase soil pH and the effect of biochar on soil pH depends on biochar production temperature and ash content of feedstock (Mukherjee et al., 2011; Slavich et al., 2013; Zhao et al., 2013). These studies were undertaken in the laboratory and there is therefore a need to investigate how biochar amendment influences the abundance of MFG under field conditions.

Soil N isotope composition ($\delta^{15}N$) is a reliable indicator of N cycling (Hietz et al., 2011; Bai et al., 2012; Ibell et al., 2013; Wang et al., 2014). When N losses occur, soils will usually become enriched in $\delta^{15}N$ (Nadelhoffer and Fry, 1994). This is because microbial processes involved in N transformations discriminate against the heavier isotope, resulting in the ^{15}N enrichment of the substrates (Criss and Criss, 1999). For example, when NH_4^+ is transformed into NO_3^-, $^{14}NH_4^+$ is preferably used which results in the enrichment of $^{15}NH_4^+$ retention in the soil (Choi and Ro, 2003). Soil N cycling can be investigated with $\delta^{15}N$ in soil–plant–biochar systems (Reverchon et al., 2014, 2015; Bai et al., 2015). In the current study, we additionally determined soil $\delta^{15}N$ of NH_4^+–N and $\delta^{15}N$ of NO_3^-–N in order to better understand the mechanisms underlying N cycling in the presence of biochar.

Biochar is usually mixed and incorporated into soil, however, macadamia develops surface feeding roots and it is not possible to incorporate biochar into soil by ploughing. Therefore, biochar was applied to the surface. The results of this experiment are valuable to different systems (e.g. agroforestry, orchards and no-till cropping systems) where it is not possible to incorporate biochar into the soil by tilling. We examined the effect of wood-based biochar on soil N cycling within the first year following biochar application in a macadamia orchard in subtropical Australia, through its influence on MFG and $\delta^{15}N$ of N species. The main objectives were to: (a) assess the effects of biochar on soil labile N dynamics, including inorganic N, $\delta^{15}N$ of NH_4^+–N and NO_3^-–N, and dissolved organic N; and (b) determine the relationships between the abundances of MFG involved in N cycling and soil chemical properties.

2. Materials and methods

2.1. Biochar characterisation

Biochar (Black Earth, Kurwongbah) was produced from pine wood chips (*Pinus* spp.) in a slow pyrolysis unit at highest treatment temperature (HTT) of 550 °C and residence time of ca 45 min. Biochar properties are summarised in Table 1.

2.2. Site description and experimental design

The experimental site was established at Beerwah in south-east Queensland, Australia (26°50′14.16″S 152°56′49.96″E), in 2012. This area is subtropical with most precipitation in summer (December–February) (Fig. 1). The soil classified as a Kurosol with an acidic pH of 5.0. Soil properties are shown in Table 2. The orchard was planted with macadamia (*Macadamia integrifolia* Maiden & Betche: Proteaceae, *variety 741*) in 2003. The experimental site was set up with a randomised complete block design with six replicates per treatment. Twenty-four plots (4 m × 4 m) were established under 24 macadamia trees, with tree at the centre. The tree spacing was 4 m × 9 m. To prevent any contamination, at each row, there was a spacing of three trees between each plot and therefore plots in each row were 12 m apart.

Biochar was surface applied nine years after macadamia planting at two rates of 10 dry t ha^{-1} (B10) and 30 dry t ha^{-1} (B30). Although biochar is often incorporated into soil using rotary tillage or ploughing, it was not possible in this orchard setting (e.g. macadamia) where soil disturbance severely damages the established root system. Before application, biochar was mixed with the soil at the ratio of 1:1.5 (w/w; dry weight) to minimise wind and rain erosion. Soil was provided from the same farm and the properties of the soil did not differ from soil collected under trees (Table 2). Each plot was divided into 16 sub-plots (1 m × 1 m) and soil and biochar were mixed for 5 min for each sub-plot and the prepared mixture was then applied homogeneously onto each sub-plot. In B10 and B30 plots, the depth of the mixture added were 1 cm and 3 cm respectively. The B10 and B30 plots received 16 kg and 48 kg dry weight biochar respectively. The control plots received the same amount of soil with no biochar, namely 10 t ha^{-1} (C10) and 30 t ha^{-1} (C30). The whole orchard was fertilised on a monthly

Table 1
Biochar characteristics and available nutrients.

pH	DI 1:25	8.21
H:C molar ratio		0.33
Ash	%	34.2
Total C	%	50.8
$\delta^{13}C$	‰	−25.1
NH_4^+–N	μg g^{-1}	14.3
NO_3^-–N	μg g^{-1}	3.82
Total N	%	0.13
$\delta^{15}N$	‰	7.71
CEC	cmol(+) kg^{-1}	44.3
Al	mg kg^{-1}	2.67
Ca	Wt%	0.77
K	Wt%	0.11
Mg	Wt%	0.031
Na	mg kg^{-1}	71.3
P	mg kg^{-1}	102.0
S	mg kg^{-1}	30.9
Zn	mg kg^{-1}	9.70
B	mg kg^{-1}	1.15
Cu	mg kg^{-1}	0.61
Fe	mg kg^{-1}	597
Mn	mg kg^{-1}	40.8

DI: Deionised water.