Aged biochar affects gross nitrogen mineralization and recovery: a $^{15}$N study in two contrasting soils

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Abstract

Biochar is a pyrolysed biomass and largely consists of pyrogenic carbon (C), which takes much longer to decompose compared to the biomass it is made from. When applied to soil, it could increase agricultural productivity through nutrient retention and changing soil properties. The biochar-mediated nutrient retention capacity depends on the biochar properties, which change with time, and on soil properties. Here, we examined the effects of a wood biochar (20 t ha$^{-1}$), that has aged (21 months) in a grassland field, on gross nitrogen (N) mineralization (GNM) and $^{15}$N recovery using a $^{15}$N tracer. A field experiment was conducted in two soil types, that is a Tenosol and a Dermosol, and also included a phosphorus (P) addition treatment (1 kg ha$^{-1}$). Compared to the control, biochar with P addition significantly increased GNM in the Tenosol. Possibly, biochar and P addition enhanced nutrient availability in this nutrient-limited soil, thereby stimulating microbial activity. In contrast, biochar addition reduced GNM in the Dermosol, possibly by protecting soil organic matter (SOM) from decomposition through sorption onto biochar surfaces and enhanced formation of organo-mineral complexes in this soil that had a higher clay content (29% vs. 8% in the Tenosol). Compared to the control, biochar significantly increased total $^{15}$N recovery in the Tenosol (on average by 12%) and reduced leaching to subsurface soil layers (on average by 52%). Overall, $^{15}$N recovery was greater in the Dermosol (83%) than the Tenosol (63%), but was not affected by biochar or P. The increased N recovery with biochar addition in the sandy Tenosol may be due to NH$_4^+$-N retention at exchange sites on aged biochar, while such beneficial effects may not be visible in soils with higher clay content. Our results suggest that aged biochar may increase N use efficiency through reduced leaching or gaseous losses in sandy soils.

Keywords: $^{15}$N recovery, aged biochar, grassland, nitrogen mineralization, phosphorus fertilization, soil properties

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Introduction

Biochar is a solid recalcitrant material that is produced through pyrolysis of biomass, and it contains predominantly pyrogenic carbon (C). When applied to soils, biochar has the potential to increase soil fertility and agricultural productivity (Jeffery et al., 2011; Biederman & Harpole, 2013) due to favourable changes in soil physical, chemical and biological properties (Mukherjee & Lal, 2013; Subedi et al., 2016). Thus, it may provide a solution to mitigating climate change through carbon-based farming. Biochar-mediated increases in productivity can manifest through nutrient retention that largely depends on its properties, including specific surface area and surface charge characteristics (Kuppusamy et al., 2016; Mia et al., 2017). Fresh biochar contains a high specific surface area and may carry a net positive surface charge (Mia et al., 2017). With time or ageing, biochar oxidizes in the soil, causing changes in its physical and chemical properties. Surface functional groups, particularly carboxylic and hydroxyl groups, are formed. As a result, negative surface charge and cation exchange capacity (CEC) of biochar increase with the ageing process (Cheng et al., 2008; Mia et al., 2017). In addition, the labile organic carbon in biochar and its intrinsic nutrient supply may be exhausted during the ageing process (Liang et al., 2014; Alotaibi & Schoenau, 2016; Wang et al., 2016). The extent of biochar ageing is also controlled by respective soil properties, such as soil organic matter (SOM), pH, nutrient status, water-holding capacity, microbial community structure and abundance, and mineralogy (Streubel et al., 2011; Ameloot et al., 2013; Fang et al., 2014). Therefore, the biochar-mediated nutrient cycling in soil may be driven by both biochar properties that change over time and the respective soil properties that regulate the ageing process.

Nitrogen (N) is a vital constituent for living organisms and often limits primary production due to its high losses through leaching, volatilization and
denitrification (Cameron et al., 2013). Application of biochar to soils can increase N retention and plant uptake (Steiner et al., 2008; Güereña et al., 2012). Several mechanisms have been proposed to explain the observed increased N retention, which include: (a) a reduction in leaching losses of NH$_4^+$ and NO$_3^-$ due to sorption, respectively, onto cation and anion exchange sites (Steiner et al., 2008; Singh et al., 2010; Clough et al., 2013; Huang et al., 2014), (b) an increase in microbial N immobilization because of biochar-mediated substrate supply or enhanced microbial growth (Bruun et al., 2011; Nelissen et al., 2012), (c) a reduction in N loss through NH$_3$ volatilization due to NH$_4^+$ adsorption to negative sites of biochar (Mandal et al., 2016), (d) a reduction in NO and N$_2$O emissions due to change in soil moisture conditions and microbial community structure (Cayuela et al., 2014), and (e) an elevated N uptake from biochar-mediated increases in plant biomass production (Steiner et al., 2008). In contrast, a reduction or neutral effects on N retention have also been reported (Bruun et al., 2012; Schomberg et al., 2012), suggesting that biochar may drive these mechanisms in the opposite directions or has no effects. These diverging results with an positive, neutral or negative impacts on N retention suggest that biochar-mediated N retention is biochar specific and may depend on its properties such as specific surface area, surface charge and fraction of labile carbon content.

Like inorganic N input, supply of N from organic sources through microbial mineralization is also a dominant source of N for plants. Nitrogen mineralization depends on a number of factors including quality and quantity of substrate, microbial community composition and abundance, soil properties and environmental conditions in soils (Kuzyakov et al., 2000; Murphy et al., 2003; Balser & Firestone, 2005; Flavel & Murphy, 2006). Fresh biochar application often increases microbial activity and decomposition of SOM, suggesting a priming effect (Zimmerman et al., 2011). As a result, gross N mineralization (GNM) usually increases with fresh biochar application (Nelissen et al., 2012; Ameloot et al., 2015; Case et al., 2015). By contrast, GNM can be reduced with biochar application for several reasons: (a) physical protection of SOM in the biochar’s pores (Lu et al., 2014), (b) enhanced microbial immobilization (Ippolito et al., 2012), and (c) enhanced sorption of NH$_4^+$ and NO$_3^-$ on biochar surface (Subedi et al., 2015). Neutral impacts of biochar addition on GNM have also been reported (Prommer et al., 2014). These inconsistent results again underscore the specificity of biochar and respective soil properties, which may interact in controlling N dynamics in soil. Additionally, biochar while aged in soils can reduce N mineralization due to (a) an exhaustion of SOM caused by a short-term positive priming effect, (b) the stabilization of SOM within biochar or by forming organo-mineral complexes (Lehmann et al., 2011; Wang et al., 2016), (c) a change in the microbial community structure and composition promoting a recalcitrant C mineralizing community (Sun et al., 2016), and (d) change in nutrient and water supplying potentials (Steiner et al., 2008).

Microbial activity and plant growth are not only affected by N supply in soils, but also by P availability, and microbes in particular have a high requirement for P (Cleveland & Liptzin, 2007). In grasslands with legume species, P supply is often more limited than N. Addition of P, therefore, may increase microbial growth and could stimulate GNM, particularly in P-limited grasslands. The GNM can be further enhanced with biochar addition as biochar has shown to increase P bioavailability through several ways. These include an increase in pH in acidic soils, promoting growth of phosphate-solubilizing bacteria and intensifying the interactions between biochar-derived organic material and fixed phosphate at mineral surfaces (Anderson et al., 2011; Biederman & Harpole, 2013; Hiemstra et al., 2013). A high level of P may also increase N recovery and utilization in soils because elevated P in soil can reduce N loss through greater plant uptake (Baral et al., 2014), although an opposite effect on N loss has also been observed (He & Dijkstra, 2015). It is not clear whether biochar, after being aged in soils, can increase GNM and contribute to N recovery, particularly when a stimulus is provided with P addition.

The aim of our study was to understand how aged biochar will affect N mineralization and $^{15}$N recovery in a grassland. We hypothesized that (a) aged biochar would increase $^{15}$N recovery, (b) P addition would positively contribute to $^{15}$N recovery, and (c) gross N mineralization would be reduced due to stabilization of SOM in organo-mineral complexes. To test these hypotheses, 21 months after a wood biochar application (20 t ha$^{-1}$), to two soil types sown with a mixture of grasses and legumes, we added a $^{15}$N tracer (0.2 g m$^{-2}$) with (0.1 g m$^{-2}$) and without P.

### Materials and methods

#### Study site and biochar field trial

The biochar field experiment was started in January, 2013, at Lansdowne Farm, Cobbitty, The University of Sydney. The details of the field experiment can be found in Keith et al. (2016). In brief, the experiment consisted of two factors, that is biochar treatments (0, 10 and 20 t ha$^{-1}$) and fertilizer applications (100% and 50% of recommended rate). The treatments were replicated four times. The same experiment was established at two sites (500 m apart) with different soil types, that is a Tenosol and a Dermosol, according to the Australian soil
classification (Isbell, 2002). The world reference base soil class of Tenosol and Dermosol is Arenosol and Cambisol, respectively. The biochar was produced from blue mallee (Eucalyptus polybractea) wood through slow pyrolysis at a maximum heating temperature of 550 °C. The basic soil and biochar properties are presented in Table 1. The biochar varied in particle size from <0.2 mm to several mm and was spread and mixed into top 10 cm soil with a tractor-driven power hoe. A mixture of grasses (Phalaris aquatica, Festuca arundinacea, Bromus wildenowii) and legumes (Medicago sativa, Medicago polymorpha, Trifolium subterraneum spp. brachycaulicum, Trifolium subterraneum ssp. subterraneum, Trifolium vesiculosum, Trifolium repens, Trifolium fragiferum, Trifolium spumosum) were sown at a planting ratio of 60% grasses and 40% legumes. Both of the soils received similar climatic exposure as they are located only 500 m apart. Mean annual minimum and maximum temperatures at the sites were 10.1 and 23.6 °C, respectively, while the average annual precipitation was 770 mm (Australian Bureau of Meteorology). The plots were irrigated regularly. After every 2 months, biomass was clipped at ground level, while soil samples were collected at two different depths, that is surface soil (0–6 cm) and subsurface soil (6–15 cm). The soils were sieved through a 4 mm sieve to separate roots from the soil, while any roots falling through the sieve were hand-picked. In each plot, plant and soil samples not labelled with 15N were also taken for determination of background levels of 15N. The surface soils (0–6 cm) were extracted with 1 m KCl (1 : 5, w/v) after shaking for 45 min. The concentration of inorganic N (NH₄NO₃) was determined on a flow injection analyser (QuickChem FIA+®, Lachat Instruments, Loveland, CO, USA; Mehnaz & Dijkstra, 2016). The NH₄NO₃ in a fraction of the soil extracts (30 mL) was collected into acidified filter paper discs (Stark & Hart, 1996). In brief, a small filter paper disc (0.5 cm in diameter) was soaked with 5 μL of 2.5 M KHSO₄ and then enveloped and sealed inside Teflon tape. The NH₄NO₃ was converted to NH₃ with 5 g of MgO and trapped with these diffusion traps for 3 days. Every day, the suspension was swirled to ensure sufficient diffusion. Next, the diffusion trap was dried on plastic stands and encapsulated into tin cups for 15N analysis. The shoot, root and soils samples were dried, ground and analysed for C and N isotopic composition and concentration using an isotope ratio mass spectrometer (IRMS) coupled to a Flash HT Plus elemental analyser (Thermo Fisher Scientific, Bremen, Germany) at the University of California, Davis, USA. Filter paper discs were also analysed for isotopic composition of N on the IRMS. Gross N mineralization was calculated according to Kirkham & Bartholomew (1954):

$$
GNM = \left[ \frac{(N_0 - N_t)}{t} \right] \times \frac{\ln(15N_0/15N_t)}{\ln(N_0/N_t)}
$$

where N₀ and Nₜ are the N concentration in soil at time zero and after 48 h, while t is the time between the two measurements (48 h). 15N₀ and 15Nₜ represent the 15N enrichment (atom%) at zero and after 48 h, respectively.

The 15N recovery in plant biomass (shoots and roots) was calculated using the following equation (Mehnaz & Dijkstra, 2016):

$$
15N_{\text{rec,plant}} = N_{\text{plant}} \times \frac{(15N_{\text{lp}} - 15N_{\text{nlp}})}{(15N_t - 15N_{\text{nlp}})}
$$

where N_{plant} and 15N_{lp} are the total N content and 15N concentration (atom%) in the labelled plant biomass, and 15N_{nlp} is the 15N enrichment of the label (atom%), while 15N_{nlp} is the average value of 15N (atom%) in nonlabelled plant biomass. The 15N recovery in soil was calculated in a similar way.

### Soil and biochar properties

Soil moisture content, pH in water (1 : 5, w/v) and electrical conductivity (Ec, 1 : 5 w/v) were determined for soil samples.
collected at all three sampling dates. The initial soil pH and EC were also determined following same methods. Additionally, at all three harvests, available P in soils was extracted with 0.03 M NH₄F-0.025 M HCl (1 : 10, w/v) and analysed colorimetrically using the ammonium paramolybdate/stannous chloride method (He & Dijkstra, 2015). The labile organic matter fraction was determined by measuring the loss of mass in ignition at 350 °C for 4 h (Mia et al., 2015). Cation exchange capacity (CEC) was measured for T3 samples using the NH₄OAc replacement method for the whole soil layer (0–15 cm; Nelissen et al., 2015). In brief, 4 g of soil was shaken for 30 min with 40 mL of 1 M NH₄OAc (pH = 7), which was repeated twice. The excess NH₄⁺ was washed three times with ethanol (70%). Next, the NH₄⁺ was replaced with Na⁺ using 40 mL 1 M NaOAc by three consecutive extractions. The solution was analysed for NH₄⁺ on a flow injection analyser as described above. The CEC of biochar was determined using similar protocol using 0.5 g of biochar. The initial CEC of soil was determined by calculating base cations replaced with 1 M ammonium acetate at pH=7.0 (Keith et al., 2016). The sand, silt and clay fractions of soils were determined by determining the density of soil suspension (1:20 m/v) in a 1 L volumetric flask. The density of silt and clay was recorded after 40 s, while the density of clay was recorded after 2 h using a hydrometer. Sodium hexa-meta-phosphate was used to disperse the soil particles. A blank reading was also taken and corrected for.

Data analysis

To test for main and interactive effects of soil type, biochar and P treatments, a full factorial analysis of variance (ANOVA) was conducted with a partial nested design, where plots were nested with soil type and considered as a random effect, while soil type, biochar and P treatments were considered as fixed factors. Soil pH, EC, NH₄⁺, NO₃⁻ and available P measured at three sampling dates were analysed using a repeated-measures ANOVA. Because the sampling date effects were never significant, ANOVA was conducted on mean values averaged across sampling dates. Biomass production and ¹⁵N recovery were analysed separately for T2 and T3. Log or reciprocal transformed data were used, when needed to fulfil the assumption of normality, while the homogeneity of variance was met for each analysis. Tukey’s HSD test was used to separate the means (*P < 0.05*). A separate one-way ANOVA was conducted for the treatment combinations, when the interactive effect was significant. Then, significant differences among the treatment combinations were assessed using Tukey’s HSD test. All analyses were performed using JMP (v 8, SAS Institute, Cary, NC, USA).

Results

Soil properties

After 21 months of biochar application, averaged across both soil types, the total C content was significantly greater (*P = 0.039*) in the plots with biochar (2.71%) compared to control plots (1.94%; Table 2). However, on average, the total soil C content for the two soil types

| Soil type                | Total C (%) | Labile organic matter (%) | Total N (%) | pH (H₂O) | EC (dS m⁻¹) | NO₃⁻ (mg kg⁻¹ soil) | Available P (mg kg⁻¹ soil) |
|--------------------------|-------------|---------------------------|-------------|-----------|-------------|----------------------|-----------------------------|
| Dermosol                 | 2.03 ± 0.13 | 3.44 ± 0.15               | 0.14 ± 0.01 | 6.84 ± 0.02 | 1003 ± 0.09 | 2.33 ± 0.2           | 224 ± 1.1                   |
| Tenosol                  | 3.13 ± 0.33 | 3.15 ± 0.21               | 0.06 ± 0.00 | 6.89 ± 0.03 | 1068 ± 0.2   | 3.86 ± 0.2           | 238 ± 0.5                   |

*The average data of T1 (1 day of ¹⁵N application), T2 (2 days after ¹⁵N application) and T3 (28 days after ¹⁵N application).*

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was similar \( (P = 0.335) \). Averaged across other treatments, the total N concentration was significantly greater \( (P < 0.001) \) in the Dermosol \( (0.14\%) \) as compared to the Tenosol \( (0.06\%) \). On average, soil pH in the Dermosol was also higher \( \text{by 0.23 units} \) than that in the Tenosol. The biochar application increased soil pH on average by 0.28 units. Overall, there was no significant main or interactive effect of soil type and biochar on inorganic N \( (\text{NH}_4^+ - \text{N} \) and \( \text{NO}_3^- - \text{N}) \). However, we found a significant interactive effect of soil type and biochar application on available P \( (P < 0.001) \) with an increased available P in the Tenosol when biochar was also applied. P addition did not affect soil available \( \text{NH}_4^+ \) or \( \text{NO}_3^- \) or available P.

**Gross N mineralization**

Averaged across all other treatments, biochar application reduced GNM by 21.2\% \( (P = 0.043) \), while P addition increased it by 47.5\% \( (P < 0.001) \), Fig. 1. However, biochar decreased GNM in the Dermosol, while it was not affected in the Tenosol \( (\text{soil type} \times \text{biochar interaction}, P = 0.024) \). Overall, P treatment increased GNM \( (<0.001) \), but the extent of increment was different for the two soil types \( (\text{soil type} \times \text{P interaction}, P = 0.017) \). A significantly greater increase in GNM with P treatment was observed in the Tenosol. In fact, there was a three-way interaction among soil type, biochar application and P addition \( (P = 0.016) \). The greatest GNM was observed in the Tenosol with both biochar and P application \( (4.67 \text{ mg N kg}^{-1} \text{ soil}) \), while the smallest GNM occurred in the same soil when only biochar was applied \( (1.85 \text{ mg N kg}^{-1} \text{ soil}) \).

**Biomass production**

Averaged across all treatments, the total biomass was significantly greater by 47\% \( (T_2) \) and 108\% \( (T_3) \) in the Dermosol than in the Tenosol \( (P = 0.013 \text{ for } T_2 \text{ and } P < 0.001 \text{ for } T_3, \text{ Fig. 2}) \). Similarly, the shoot biomass was greater by 200\% at \( T_2 \) and by 369\% at \( T_3 \) in the Dermosol compared to the Tenosol \( (P < 0.001) \). At \( T_3 \), there was a significant interactive effect among soil type, biochar application and P addition on shoot biomass production \( (P = 0.046) \). In the Tenosol, P addition increased shoot biomass production without biochar, but decreased it with biochar, while in the Dermosol, P addition decreased shoot biomass production without biochar, but increased it with biochar. Averaged across the other treatments, biochar did not affect shoot biomass production in either of the harvests, but biochar increased root biomass by 30\% at \( T_3 \) \( (P = 0.003) \).

**Total \( ^{15} \text{N} \) recovery**

Averaged across all other treatments, total \( ^{15} \text{N} \) recovery was significantly greater in the Dermosol compared to the Tenosol, both at \( T_2 \) and \( T_3 \) \( (P < 0.001, \text{ Fig. 3}) \). There was no overall biochar and P treatment effect at either of the harvests \( (P > 0.05) \). But, we found an interactive effect of soil type and biochar application at both harvests \( (P = 0.02 \text{ for } T_2 \text{ and } P = 0.01 \text{ for } T_3) \). At \( T_3 \), biochar increased total \( ^{15} \text{N} \) recovery by 21\% in the Tenosol, while it did not affect the recovery in the Dermosol. At \( T_2 \), there was a significant interaction between biochar and P treatments \( (P = 0.037) \), where combined application of biochar and P reduced \( ^{15} \text{N} \) recovery by 8\% in comparison with the control. At \( T_3 \), the three-way interaction among soil type, biochar application and P addition was significant \( (P = 0.021) \). Biochar treatment increased \( ^{15} \text{N} \) recovery in the Tenosol when P was not supplied, but a similar effect was not evident in the Dermosol.

\( ^{15} \text{N} \) recovery in plants

Averaged across all other treatments, the total plant \( ^{15} \text{N} \) recovery was significantly greater in the Dermosol than...
that in the Tenosol at both harvests ($P < 0.001$, Fig. 4). Compared to the Tenosol, the recovery was 86% and 39% higher in the Dermosol at T2 and T3, respectively. Across the other two treatments, biochar application had a negative effect on $^{15}$N recovery in plants at T2 ($P < 0.001$) with an overall reduction of 21%. Overall, P treatment did not affect $^{15}$N recovery in plants at either of the harvests. At T3, both two-way (soil type x P addition interaction, $P = 0.015$) and three-way interactions (soil type x biochar x P addition interaction, $P = 0.003$) were significant. Compared to control (without biochar and P), biochar application increased total plant $^{15}$N recovery by 22% in the Tenosol without P addition but such effect was not evident in the Dermosol.

On average, the shoot $^{15}$N recovery was greater in the Dermosol compared to the Tenosol at both T2 and T3 ($P < 0.001$, Fig. 4). The increments were 119% at T2 and 70% at T3. At T2, biochar had an overall negative effect on shoot $^{15}$N recovery ($P = 0.001$). All other treatments or interactions were not significant. There was no overall effect of soil type, biochar and P treatment on root $^{15}$N recovery at either of the harvests. But, at T2, the soil type x biochar interaction was significant ($P = 0.03$) with a negative effect of biochar in the Tenosol, but not in the Dermosol. At T3, the soil type x P interaction was significant ($P = 0.02$) with significant reduction in $^{15}$N recovery in roots with P addition in the Tenosol, but not in the Dermosol.

$^{15}$N recovery in soil

Averaged across all other treatments, the total soil $^{15}$N recovery (0–15 cm) was greater by ~28% in the Tenosol at T2, while it was greater by ~43% in the Dermosol at T3 ($P < 0.001$, Fig. 3). Overall, biochar had a positive effect on $^{15}$N soil recovery at T2 ($P = 0.006$), but this was not evident at T3 ($P = 0.256$). But, at both harvests, the soil type x biochar application interaction was significant. Biochar application increased soil $^{15}$N recovery (by 36% at T2 and by 57% at T3) in the Tenosol, but it did not have an effect in the Dermosol. Phosphorus treatment and other interactive effects were not significant in either of the harvests.

The $^{15}$N recovery in the subsurface soil (6–15 cm, Fig. 5) was generally much smaller (overall ~0.05%) than that in the surface soil (0–6 cm). At T2, none of the treatments or their interactions were significant except for the interactive effect of biochar and P treatment at T2 ($P = 0.042$). At T3, the overall $^{15}$N recovery in the

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subsurface soil was greater in the Tenosol than in the Dermosol ($P < 0.001$). In fact, we observed a significant soil type × biochar interaction ($P = 0.041$). The Tenosol receiving biochar had a lower subsoil $^{15}$N recovery compared to the Tenosol without biochar, while there were no biochar effects in the Dermosol.

**Discussion**

**Aged biochar effect on N mineralization is soil specific**

The GNM rate in our study ranged between 1.85 and 4.67 mg N kg$^{-1}$ soil day$^{-1}$, which is consistent with several other studies (1–10 mg N kg$^{-1}$ soil day$^{-1}$; Cheng et al., 2012; Gómez-Rey & González-Prieto, 2015), but it is greater than the rate (0.73 mg N kg$^{-1}$ soil day$^{-1}$) observed by Nelissen et al. (2015). Biochar reduced GNM in the Dermosol but not in the Tenosol (Fig. 1), suggesting a soil-specific effect of biochar. Biochar-mediated reduction in GNM suggests that biochar decreased SOM decomposition indicating a negative priming effect, which has been reported in a number of studies (Kimetu & Lehmann, 2010; Fang et al., 2014, 2015; Keith et al., 2015; Hernandez-Soriano et al., 2016). In addition, Nelissen et al. (2015) found an increased GNM just after biochar application in an incubation study with a maize field soil, but this effect disappeared after 1 year. A biochar-mediated reduction in GNM in the Dermosol may not be due to substrate limitation (Luo et al., 2011; Wang et al., 2016) as we found an increased labile organic matter in the soils with biochar (Table 2). Therefore, stabilization of SOM by biochar through sorption that rendered SOM inaccessible to microbial decomposition may have caused a reduction in GNM (Lehmann et al., 2011). The stabilization process in the Dermosol, relatively rich in clay (Table 1), may be further enhanced with aged biochar, which carries an increase in surface acidic functional groups (Liang et al., 2010; Hernandez-Soriano et al., 2016). In fact, in our experiment, we found an increased CEC in soils with biochar, suggesting that part of the biochar was oxidized. Additionally, biochar intrinsically contained a considerable CEC (51 cmol$_c$ kg$^{-1}$). Therefore, we suggest the stabilization of SOM through sorption onto biochar and further protection through formation of organo-mineral complexes caused a negative effect on GNM in the Dermosol (Fang et al., 2014). In addition to this, as the Dermosol was rich in clay content (29%) and clay has higher surface area, SOM possibly was strongly adsorbed onto the surface area, thus becoming

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unavailable for microbes. It is further possible that the decrease in GNM with biochar addition in the Dermosol was caused by a change in microbial community structure (Lehmann et al., 2011), but unfortunately, we did not analyse the microbial community structure in the soils.

Overall, biochar did not affect GNM in the Tenosol. However, GNM was accelerated and occurred at the greatest rate (4.67 mg N kg\(^{-1}\) soil day\(^{-1}\)) when both biochar and P were applied (Fig. 1). This result suggests that the microbial community in the Tenosol had greater access to SOM, but may be more limited by P than in the Dermosol. The exceptional increase in GNM in the biochar-amended Tenosol with P addition suggests that such a combination may have enhanced additional supply of nutrients to the microbes that triggered decomposition of SOM and GNM. Furthermore, the Tenosol had a much smaller clay content than the Dermosol (Table 1), and therefore, biochar may have had limited possibility of forming organo-mineral complexes in the Tenosol.

**Aged biochar increases \(^{15}\text{N}\) recovery in the Tenosol**

Averaged across all other treatments, the total \(^{15}\text{N}\) recovery was significantly greater in the Dermosol...
(83%) compared to the Tenosol (63%). The elevated recovery in the Dermosol resulted from an increased recovery in plants (overall 58% compared to 37% in the Tenosol) as the Dermosol was more productive (on average 79% greater biomass production) than the Tenosol. Compared to the Dermosol, the Tenosol has less clay content (8%). Therefore, the loss of 15N through leaching and gaseous emissions may be high in the Tenosol (Keith et al., 2016). Biochar application increased total 15N recovery in the Tenosol on average by 12%, while the biochar effect was not evident in the Dermosol. The elevated recovery of 15N after biochar application was caused by an increased retention of N in the soil (Fig. 3). Aged biochar can increase NH₄⁺-N retention at cation exchange sites (Steiner et al., 2008; Huang et al., 2014). For similar reasons, we propose that the observed greater 15N retention in the Tenosol was caused by adsorption of 15NH₄⁺-N (Steiner et al., 2008; Ding et al., 2010; Singh et al., 2010). Although biochar pores are usually blocked with soil particles and SOM after soil application, in the Tenosol, biochar may carry some specific surface area, because this soil was sandy (52%) and low in organic matter content. Therefore, the biochar’s surfaces may have contributed partly to the sorption of 15NH₄⁺-N and 15NO₃⁻-N. The greater retention of NH₄⁺-N on the exchange sites, derived from biochar in the Tenosol, may also have caused reduced leaching and gaseous N losses. As a measure of N leaching, we measured 15N recovery in the subsurface soil (6–15 cm) and indeed found a significantly reduced amount of labelled N in the Tenosol with biochar addition (Fig. 5). Additionally, a reduction in NH₄⁺-N and NO₃⁻-N leaching may have resulted from the significantly greater soil moisture retention in biochar-applied plots (Table 2). Although we found a decreased downward movement of 15N with biochar in the Tenosol, the fraction of 15N recovered in the subsurface soil was relatively small (~0.05%) in comparison with the total soil recovery (~14%). Therefore, we do not expect that reduced leaching is the main driver for the biochar-mediated increased 15N recovery in the Tenosol. Instead, biochar-mediated reduction in gaseous N losses may be more important. Biochar may have reduced NH₃ volatilization directly after the 15N injection due to increased adsorption of NH₄⁺-N (Guimarães et al., 2015; Mandal et al., 2016), provided no stimulation was triggered from a biochar-mediated pH increase (only ~0.3 unit increase for this study, Table 2; Schomberg et al., 2012; Zhao et al., 2013). We found a slightly increased NH₄⁺-N concentration (but not significant) after 2 days of 15N injection in the Tenosol receiving biochar, suggesting that biochar-mediated reduction in NH₃ volatilization might have been small. Biochar addition also had no effect on the N₂O emission during the first 2 years of the experiment (Keith et al., 2016). Therefore, it is likely that aged biochar reduced loss of N through other gases (e.g. NO and N₂).

Averaged across soil types, biochar increased the CEC but a biochar-mediated increase in 15N recovery was not found in the Dermosol. The CEC was relatively high in this soil (Table 2), and therefore, the biochar-mediated increase in CEC may have contributed little to the soil 15N recovery in the Dermosol. Further, the high biomass production in the Dermosol took up most of the 15N (on average ~62% recovered in plant), therefore decreasing the possibility of 15N to be lost through leaching or as gaseous N, while biochar did not significantly increase biomass production in this soil type (Fig. 2, Keith et al., 2016).

Overall, P addition did not affect 15N recovery but the biochar-mediated increased 15N recovery in the Tenosol at T₃ disappeared in the presence of P, while the recovery was unaffected by both biochar and P treatments in the Dermosol (causing the soil types × biochar × P interaction; Fig. 2b). P addition also reduced 15N recovery in a pot experiment grown with a C3 grass and legume (He & Dijkstra, 2015). It was suggested that activity of nitrifiers and denitrifiers could be stimulated with P addition, thereby enhancing the loss of gaseous N, which has also been observed by others (Mori et al., 2010; Wang et al., 2014; Mehnaz & Dijkstra, 2016). It is unclear why P addition only reduced 15N recovery in the Tenosol with biochar, but possibly microbial activity, including the activity of nitrifiers and denitrifiers, was increased with biochar application. Further investigation is required to understand the role of P for nitrifiers and denitrifiers and the consequences for N loss and retention.

Our results showed that aged biochar decreased gross N mineralization in the Dermosol with relatively greater clay content, while it had an opposite effect in the nutrient-poor and sandy-textured Tenosol, particularly when P was also applied. Biochar application may increase NH₄⁺-based fertilizer use efficiency in nutrient-poor sandy soils due to an increased retention of N on the exchange sites associated with CEC of biochar. In addition, biochar surface area may have contributed partly to the increased retention. However, the biochar effects on NH₄⁺ retention may be less effective in soils that already have adequate exchange sites for N retention.

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References
Atalib K, Schoenau J (2016) Application of two bioenergy by-products with contrasting carbon availability to a Prairie soil: three year crop response and changes in soil biological and chemical properties. Agronomy, 6, 13.
Ameloot N, Graber ER, Verheijen FGA, de Neve S (2013) Interactions between biochar stability and soil organisms: review and research needs. European Journal of Soil Science, 64, 379–390.
Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR, Ameloot N, Sleutel S, Das KC, Kammann C, M, Bauer P, Mollemann B (2013) Natural and pyrogenic humic acids at goethite and natural oxide surfaces interacting with phosphate. Environmental Science and Technology, 47, 9182–9189.
Huang M, Yang L, Qin H, Jiang L, Zou Y (2014) Fertilizer nitrogen uptake by rice increased by biochar application. Biology and Fertility of Soils, 50, 997–1000.
Ippolito JA, Novak JM, Busscher WJ, Ahmeda M, Rehrah D, Watts DW (2012) Switchgrass biochar affects two aridisols. Journal of Environmental Quality, 41, 1123–1130.
Isbell R (2002) The Australian Soil Classification, Vol. 4. CSIRO Publishing, Collingwood, VIC.
Jeffery S, Verheijen FGA, van der Velde M, Bastos AC (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment, 144, 175–187.
Keith A, Singh B, Dijkstra FA, van Ogtrop F (2015) Biochar reduces the rhizosphere priming on soil organic carbon. Soil Biology & Biochemistry, 88, 372–379.
Keith A, Singh B, Dijkstra FA, van Ogtrop F (2016) Biochar field study: greenhouse gas emissions, productivity, and nutrients in two soils. Agromony, 108, 1–11.
Kimetu JM, Lehmann J (2011) Stability and stabilization of biochar and green manure in soil with different organic carbon contents. Australian Journal of Soil Research, 49, 577.
Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Journal, 18, 33.
Kuppusamy S, Thavampani P, Megharaj M, Venkateswarlu K, Naidu R (2016) Agro-nomic and remedial benefits and risks of applying biochar to soil: current knowledge and future research directions. Environment International, 87, 1–12.
Kryzyskov V, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry, 32, 1485–1498.
Lehmann J, Rillig MC, Theis J, Massiello CA, Hockaday WC, Crowley D (2011) Biochar affects on soil biota—a review. Soil Biology and Biochemistry, 43, 1812–1836.
Liang B, Lehmann J, Seh S, et al. (2010) Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry, 41, 206–213.
Liang F, Li G, Lin Q, et al. (2014) Crop yield and soil properties in the first 3 years after biochar application to a calcareous soil. Journal of Integrative Agriculture, 13, 525–532.
Lu W, Ding W, Zhang J, Li Y, Bolan N, Xie Z (2014) Biochar suppressed the decomposition of organic carbon in a cultivated sandy loam soil: a negative priming effect. Soil Biology and Biochemistry, 76, 12–21.
Luo Y, Durencamp M, de Nobili M, Liu Q, Brookes PC (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. Soil Biology & Biochemistry, 43, 2304–2314.
Mandal S, Thangarajan R, Bolan NS, Sarkar B, Khan N, Ok YS, Naidu R (2016) Biochar-induced concomitant decrease in ammonia volatilisation and increase in nitrogen use efficiency by wheat. Chemosphere, 142, 120–127.
Mehnaz KR, Dijkstra FA (2016) Denitrification and associated N₂O emissions are limited by phosphorus availability in a grassland soil. GCB Bioenergy, 25, 34–41.
Mai S, Eddin N, Hossain SA, Amim R, Meta FZ, Haemstra T (2015) Production of biochar for soil application: a comparative study of three kiln models. Pedosphere, 25, 696–702.
Mai S, Dijkstra FA, Singh B (2017) Long-term ageing of biochar: a molecular understanding with agricultural and environmental implications. Advances in Agronomy, 141, 1–51.
Men T, Ohba S, Ishizaka S, Konda R, Wicaksono A, Hariyanto J, Hardjono A (2010) Effects of phosphorus addition on N₂O and NO emissions from soils of an Acacia mangium plantation. Soil Science and Plant Nutrition, 56, 782–788.
Mukherjee A, Lal R (2013) Biochar impacts soil physical properties and greenhouse gas emissions. Agronomy, 3, 313–339.
Murphy D, Recous S, Stockdale E, Favery IR, Jensen L, Hatch D, Goulding KW (2003) Gross nitrogen fluxes in soil: theory, measurement and application of ¹⁵N pool dilution techniques. Advances in Agronomy, 79, 69–118.
Nelissen V, Rütting T, Huyngh D, Stadler J, Ruysschaert G, Boeckx P (2012) Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biology and Biochemistry, 55, 20–27.
Nelissen V, Rütting T, Huyngh D, Ruysschaert G, Boeckx P (2015) Temporal evolution of biochar’s impact on soil nitrogen processes – a ¹⁵N tracing study. GCB Bioenergy, 7, 635–645.
Prommer J, Wanek W, Hofhansl F et al. (2014) Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. *PLoS ONE*, 9, e86388.

Schomberg HH, Gaskin JW, Harris K et al. (2012) Influence of biochar on nitrogen fractions in a coastal plain soil. *Journal of Environmental Quality*, 41, 1087.

Singh BP, Hatton BJ, Cowie AL, Kathuria A (2010) Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of Environmental Quality*, 39, 1224.

Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal*, 60, 1846.

Steiner C, Glaser B, Teixeira GW, Lehmann J, Blum WEH, Zech W (2008) Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. *Journal of Plant Nutrition and Soil Science*, 171, 893–899.

Streubel JD, Collins HP, Garcia-Perez M, Tarara J, Granatstein D, Kruger CE (2011) Influence of contrasting biochar types on five soils at increasing rates of application. *Soil Science Society of America Journal*, 75, 1402.

Subedi R, Kammann C, Pelissetti S, Taupe N, Bertora C, Monaco S, Grignani C (2015) Does soil amended with biochar and hydrocarbon reduce ammonia emissions following the application of pig slurry? *European Journal of Soil Science*, 66, 1044–1053.

Subedi R, Taupe N, Ikoyi I et al. (2016) Chemically and biologically mediated fertilizing value of manure-derived biochar. *Science in the Total Environment*, 530, 924–933.

Sun D, Meng J, Xu GE, Chen W (2016) Microbial community structure and predicted bacterial metabolic functions in biochar pellets aged in soil after 34 months. *Applied Soil Ecology*, 100, 135–143.

Wang F, Li J, Wang X, Zhang W, Zou B, Neher DA, Li Z (2014) Nitrogen and phosphorus addition impact soil N₂O emission in a secondary tropical forest of South China. *Scientific Reports*, 4, 5615.

Wang J, Xiong Z, Kuzyakov Y (2016) Biochar stability in soil: meta-analysis of decomposition and priming effects. *GCB Bioenergy*, 8, 512–523.

Zhao X, Yan X, Wang S, Xing G, Zhou Y (2013) Effects of the addition of rice-straw-based biochar on leaching and retention of fertilizer N in highly fertilized cropland soils. *Soil Science and Plant Nutrition*, 59, 771–782.

Zimmerman AR, Gao B, Ahn M-Y (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry*, 43, 1169–1179.