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Biochar carbon dynamics in physically separated fractions and microbial use efficiency in contrasting soils under temperate pastures

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ABSTRACT

There is overwhelming evidence for the long-term persistence of biochar in soil. However, the partitioning of biochar into light and heavy carbon (C) fractions and microbial biomass C (MBC), and the dynamics of C use efficiency (CUE_E: net incorporation of biochar into MBC per unit of biochar-C consumed, including microbial death and recycling of biochar-derived microbial metabolites) in planted soil systems are poorly understood. A ^{13}C-labelled wood biochar ($\delta^{13}C$: −36.7‰) was incorporated into topsoil (0–10 cm) in an Arenosol, Cambisol and Ferralsol under C_3 dominated temperate pastures ($\delta^{13}C$: −25 to −27‰). The partitioning of biochar-C into the various soil C pools and CUE_E were measured at 4, 8 and 12 months. The results showed that 8.6–28.2% of the biochar-C in the top soils was distributed to the heavy fraction (HF) within 4 months, which increased to 11.0–33.3% at 8 and 12 months. Biochar-C recovery in the HF was the highest in the Ferralsol (*cf.* Arenosol and Cambisol), possibly due to greater interaction of biochar and biochar-derived microbial metabolites with soil minerals. Biochar significantly increased MBC across the three soils. Biochar-derived MBC ranged from 22 to 93 mg C kg^{-1} soil over time (Arenosol < Cambisol < Ferralsol), representing 11–20% of the total MBC pool. Biochar CUE_E was 0.20–0.27 at 4 months, which decreased over time, possibly due to lowering of biochar-C availability to microbes. Further, although biochar-derived MBC was higher, biochar CUE_E was lower in the Ferralsol (*cf.* Arenosol and Cambisol), likely supported by higher microbial respiration and turnover, and lower recycling of microbial metabolites *via* greater organo-mineral interaction. Here, the study advanced our understanding of key C cycling processes, such as CUE_E and the temporal fate of biochar-derived C in an organo-mineral fraction with relevance for biochar sequestration in contrasting soils under planted field conditions.

1. Introduction

Biochar is a form of pyrogenic carbon (PyC) produced *via* the pyrolysis of biomass under controlled conditions (IBI, 2012). Biochar has been used as a potential soil amendment with aims to improve soil quality (such as water retention and nutrient availability) and to promote agronomic benefits (Glaser et al., 2002; Chan et al., 2007; Steinbeiss et al., 2009; Lehmann and Joseph, 2015). Biochar produced at higher temperatures (*i.e.* > 400 °C) tends to have higher aromaticity than at lower temperatures, giving a centennial-scale residence time in soil (Lehmann et al., 2006; Major et al., 2010; Haefele et al., 2011; Knoblauch et al., 2011; Singh et al., 2012). Although carbon (C) in biochar is chemically recalcitrant (Singh et al., 2012; Harvey et al., 2016), biochar can be degraded and released as CO_2 (generally defined as “mineralisation”), while being altered to other organic substances by abiotic and biotic processes in soils, typically microbial metabolites and residues (defined as “decomposition”) (Lehmann et al., 2015). It has been reported that biochar-C mineralisation can be affected by site-specific soil properties (Luo et al., 2011; Singh et al., 2015), temperature [*e.g.* (sub)tropical *vs.* temperate environment] (Zimmermann et al., 2012; Fang et al., 2014b) and water availability (Nguyen and Lehmann, 2009). Despite the existing research on biochar stability in soil, understanding of the fate of biochar in different soil C fractions including the microbial biomass pool is still lacking, particularly under planted field conditions. However, acquiring the knowledge of fate of biochar *in-situ* in various C pools under different climate regimes is critical for a better understanding of the mechanisms that promote C sequestration.

Development of organo-mineral complexes through processes such as ligand exchange, cation bridging, and van der Waals' interactions has been suggested to be an important mechanism for the persistence of

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organic matter in soil (von Lützow et al., 2006). To assess whether organic material is potentially stabilised in soil, a density fractionation method was proposed to separate the "heavy fraction" (density: > 1.6–2.0 g cm^{-3}) containing organo-mineral complexes and the "light fraction" (density: < 1.6–2.0 g cm^{-3}) comprising mineral-free soil organic C (SOC) (Golchin et al., 1994; Glaser et al., 2000; Sohi et al., 2001; Herath et al., 2014; Singh et al., 2014a; Weng et al., 2017). Glaser et al. (2000) found a high proportion of PyC stabilisation from *Terra preta* soils of the Amazon in the heavy fraction where PyC was partly embedded within plaques of Fe- and Al- oxides on the mineral surface, although the majority of PyC was still recovered in the light fraction. Due to the existence and development of oxygen functional groups during degradation, biochar could interact with mineral surfaces (Fe-, Al-, Mn-oxides, and phyllosilicates) and dissolved metal ions (such as Ca^{2+}, Al^{3+} and Fe^{3+}) to form organo-mineral complexes (Gu et al., 1994; Baldock and Skjemstad, 2000; Lin et al., 2012; Qayyum et al., 2012; Lehmann et al., 2015). Biochar-C stability could be affected by the degree of organo-mineral interaction, which relates to the clay content and clay mineral composition of soils. For example, Fang et al. (2015) reported that biochar-C mineralisation in varied soils followed the order of clay-rich Oxisol (Ferralsol) < clay-rich Vertisol < clay-poor Inceptisol (Arenosol). Despite similar clay content in the Ferralsol and Vertisol, the lower biochar-C mineralisation occurred in the Ferralsol, possibly due to greater proportions variable charged minerals (goethite, hematite, gibbsite and kaolinite *etc.*), which can have greater interactions with C, than the permanently charged mineral (smectite), which predominated in the Vertisol (Fang et al., 2015). However, the extent and dynamics of biochar and mineral interactions in soils are far from understood (Herath et al., 2014; Singh et al., 2014b), especially in different soil types under realistic field conditions.

While the sequestration potential of PyC or biochar-C in soil has received great attention (Lehmann et al., 2015), the processing of biochar by soil microorganisms, which regulates biochar dynamics in soil (Luo et al., 2013; Lehmann et al., 2015), is still not clear. An important parameter which integrates the processes of microbial metabolism at ecosystem-scale is C use efficiency (CUE_E), defined as the ratio of net microbial growth over substrate consumption, and substrate consumption is the sum of microbial production, total respiration and death (Geyer et al., 2016). In other words, CUE_E over a longer period (several days to months) encompasses the effects of microbial turnover and some other ecosystem processes, such as soil chemical and biological properties and environmental factors, on the synthesis of substrates and the recycling of microbial metabolites (*i.e.* necromass and exudates) (Geyer et al., 2016). For example, products of microbial metabolism (such as microbial cell wall envelopes) would associate with soil mineral surfaces, act as binding agents, and incorporate into aggregates (Lehmann and Kleber, 2015), leading to further stabilisation of microbial metabolites into stable SOC pools (Kiem and Kögel-Knabner, 2003; Marschner et al., 2008; Knicker, 2011; Miltner et al., 2012). Hence, the recycling of biochar-derived microbial metabolites could be constrained by interactions with soil minerals, thus impacting biochar-derived microbial CUE_E over time (Geyer et al., 2016). Therefore, monitoring of CUE_E from field trials will illustrate the fate of biochar-derived microbial metabolites, and the role of soil properties in stabilising these metabolites.

Recent studies have shown that microorganisms utilise only a small proportion of biochar. For instance, Luo et al. (2013) reported that less than 0.15% of added biochar-C was incorporated into microbial biomass C after 87 days of laboratory incubation. Kuzyakov et al. (2014) found that between 0.30 and 0.95% of initial labelled biochar-C recovered in microbial biomass after the 3.5 years of laboratory incubation. Singh et al. (2014b) reported 0.14–0.18% of biochar-C incorporated into microbial biomass after 10 months under field conditions. Although some studies have made field-based assessment of biochar stability (Singh et al., 2015; Weng et al., 2015) and stabilisation of rhizodeposits (Weng et al., 2017), we are unaware of any studies that have assessed microbial CUE_E of biochar in systems where plants are present. Since the input levels of rhizodeposits could increase microbial activity (Blagodatskaya et al., 2014), plant productivity differences may interact with soil properties to influence biochar-derived microbial biomass C (MBC) and CUE_E. This knowledge is important to better understand mechanisms of microbial processing and sequestration of biochar-C in contrasting soils under different temperate pastures.

The current study aims to provide insights into *in-situ* temporal dynamics of wood-derived biochar-C into: (i) the mineral-free and mineral-associated organic fractions; (ii) biochar-C incorporation into the microbial biomass pool; and (iii) biochar CUE_E by microorganisms in contrasting soils with varying plant productivity, SOC content, clay content and mineralogy under temperate pasture systems. A ^{13}C-depleted *Eucalyptus saligna* biochar ($\delta^{13}C$: −36.7‰) was used that allowed assessment of biochars fate within soil physical fractions (mineral-free light and mineral-associated heavy C) and microbial biomass after application in an Arenosol, Cambisol or Ferralsol ($\delta^{13}C$: −25 to −27‰) across two different temperate climatic conditions and over a period of 12 months. Based on the findings reported in the current literature, we hypothesised that:

(i) The incorporation of biochar into a mineral-associated organic fraction will be dominantly affected by clay content and mineralogy of the soils, rather than variations in other site-specific characteristics (such as pasture species, productivity and climate);
(ii) Total and biochar-derived MBC will be higher in the C-rich soils (Ferralsol and Cambisol) with relatively high pasture growth compared to the C-poor Arenosol with low pasture growth; and
(iii) Biochar CUE_E will be lower in the Ferrosol and Cambisol, due to greater biochar-C mineralisation and/or limited accessibility of biochar and biochar-derived metabolites in a mineral-associated organic fraction, relative to the Arenosol.

2. Material and methods

2.1. Field experiments

The experimental field sites for the current study were located at (i) Cobbitty, New South Wales (NSW) and (ii) Elliott, Tasmania, Australia. The soils were classified as an Arenosol and a Cambisol at Cobbitty (−34.02340°, 150.66350°), and a Ferralsol at Elliott (−41.08110°, 145.77035°) as per the World Reference Base (Soil Survey Staff, 2010). The climate at the Cobbitty site is mild temperate, with a mean annual temperature of 17.2 °C, and a mean annual rainfall of 690 mm. The climate at the Elliot site is cool temperate, with a mean annual temperature of 12.3 °C, and a mean annual rainfall of 988 mm (Table 1).

Biochar was produced at 450 °C by slow pyrolysis (*e.g.* 5–10 °C min^{-1} from 20 to 450 °C, 40 min residence time) from a $\delta^{13}C$-depleted (−37.7‰) *Eucalyptus saligna* biomass, comprising stem wood, branches/twigs, and leaves. The *Eucalyptus* trees had been grown in an elevated, CO_2 environment for two years. At each site, there were four biochar-amended micro-plots and four control (non-amended) micro-plots of 0.66 m diameter each in a selected pasture area of approximately 13 m length and 7 m width (Singh et al., 2015). To establish the circular micro-plots, the areas were marked with a ring. Soil at 0–10 cm depth was excavated and then mixed with biochar at 29.2 t ha^{-1}, or mixed without biochar (*i.e.* for the control). Soil at the 10–12 cm depth was also excavated followed by lining of the perimeter of the micro-plots with plastic garden edging (15 cm wide; 4 mm thick) to 12 cm depth. The micro-plots were then back-filled, firstly with the soil excavated from the 10–12 cm depth and then with the biochar-soil mixture (biochar micro-plots) or the mixed soil (control micro-plots). Bulk density of biochar was 0.33 g cm^{-3}, which was lower than the soil bulk density (Table 1). Hence, surplus soil was removed from the 10–12 cm layer during backfilling and the soil with or without biochar was compacted gently to achieve close to original bulk density of the