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Short communication

Does biochar interfere with standard methods for determining soil microbial biomass and phenotypic community structure?

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ABSTRACT

Due to its high sorption affinity for organic compounds, biochar may interfere with extraction procedures involving such compounds used for microbially-related assays commonly applied to soils. Here we assessed the impact of two biochars (derived from pine bark and produced at 300 and 600 °C) at three concentrations (0, 12.5, and 50 g kg^{-1}) in three distinct arable soils with contrasting textural classes (loamy sand, sandy loam, and clay) on the determination of soil microbial biomass C by fumigation–extraction, fungal biomass by ergosterol analysis, and microbial community structure as defined by phospholipid fatty acid (PLFA) profiling. Biochar did not affect the apparent concentration of soil microbial biomass C and had no significant impact on apparent PLFA profiles. By contrast, the apparent extraction efficiency of ergosterol was affected dependent on soil type, biochar production temperature, and biochar concentration. Nonetheless, ergosterol contents of biochar-amended soils can be accurately estimated by correcting for reduced recovery using an ergosterol spike.

Biochar, a loosely-defined C-rich solid product derived from biomass pyrolysis and intended for use as a soil amendment, is believed to be a highly stable material that may aid in climate change mitigation through C sequestration, while enhancing soil fertility and quality (e.g. Lehmann, 2007). However, prior to its widespread production and application, more research is needed to avoid unintended environmental consequences (Kookana et al., 2011). In particular, it is of importance to understand the effects of biochar on soil microorganisms, inasmuch as they play a critical role in key soil processes such as organic matter transformation and nutrient cycling.

Extraction of organic compounds from soils are the basis of many important microbial determinations, such as microbial biomass C using the fumigation–extraction procedure (Vance et al., 1987), the quantification of the fungal biomass using ergosterol as a specific biomarker (Ruzicka et al., 1995), and the characterization of the microbial community structure using phospholipid fatty acid (PLFA) analysis (Frostegård et al., 1993). Biochar has a very high surface area and sorption affinity for a plethora of organic compounds, making them less bioavailable (Smernik, 2009). For this reason, we hypothesised that biochar may interfere with extraction procedures used for microbial measurements (Lehmann et al., 2011), thereby hampering the study and meaningful interpretation of the effects of biochar on soil microorganisms. Supporting this, Gomez et al. (2014) observed significant decreases in the extraction efficiency of a recovery PLFA standard with increasing biochar application rates above 5%. Nonetheless, the only systematic study specifically addressing this issue was conducted by Durenkamp et al. (2010), who tested the efficiency of microbial biomass C and ninhydrin-reactive N estimations using the fumigation–extraction (Brookes et al., 1985; Vance et al., 1987) method in soils that had been amended with biochar and activated charcoal. They concluded that the addition of biochar did not decrease the extraction efficiency of soil microbial biomass C. Our objective was to verify this observation in a wider range of soils and to

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Table 1
Analysis of variance for extractable C from non-fumigated and fumigated soils, microbial biomass C, ergosterol recovery, ergosterol content, and first (PC1), second (PC2), and third components (PC3) derived from PLFA data, as affected by soil type (SO), biochar production temperature (BT), and biochar concentration in soil (BC).

Source	Extractable C from non-fumigated	Extractable C from fumigated soils	Microbial biomass C	Ergosterol recovery	Ergosterol content	PLFA PC1	PLFA PC2	PLFA PC3
SO	***	***	***	***	***	***	***	**
BT	NS	**	NS	*	NS	NS	NS	NS
BC	NS	NS	NS	***	NS	NS	***	**
SO × BT	**	*	**	NS	NS	NS	NS	NS
SO × BC	NS	NS	NS	NS	NS	NS	NS	NS
BT × BC	NS	NS	NS	***	NS	NS	NS	NS
SO × BT × BC	NS	NS	NS	*	NS	NS	NS	NS

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS $P > 0.05$

establish if the presence of biochar in soil samples interferes with explicitly lipid-based assays such as PLFA profiles or ergosterol.

We conducted a full factorial experimental design (three replicates) involving two biochars incorporated into three arable soils at rates of 0, 12.5, and 50 g kg^{-1}. The chars were prepared by heating pine bark in a pyrolysis furnace at 300 or 600 °C for 2 h. The resultant biochar samples were ground to pass through a 0.5-mm sieve. The biochar produced at 300 °C had a pH of 6.7, C content 662 g kg^{-1}, N content 3.3 g kg^{-1}, and molar H/C ratio 0.7, whereas that at 600 °C had a pH of 8.7, C content 815 g kg^{-1}, N content 3.3 g kg^{-1}, and molar H/C ratio 0.3.

Soil samples (15 cm depth) of contrasting texture were collected from Cranfield University Farm at Silsoe (UK: LAT 52°00′20.3″N; LONG 0°25′41.9″W), derived from three soil series, viz. a loamy sand (Cottenham Series, 83.0% sand, 10.8% silt, 6.2% clay, pH in water 7.3, 12.4 g kg^{-1} organic C, 1.2 g kg^{-1} total N), a sandy loam (Maplestead Series, 78.6% sand, 10.8% silt, 10.5% clay, pH in water 7.4, 14.7 g kg^{-1}organic C, 1.4 g kg^{-1} total N), and a clay (Denchworth Series, 46.0% sand, 23.8% silt, 30.2% clay, pH 7.0, 29.1 g kg^{-1}organic C, 3.9 g kg^{-1} total N). Field-moist samples were homogenized and sieved to 2 mm.

Prepared soils were thoroughly mixed with the appropriate quantity of biochar by hand stirring immediately before microbiological determinations. For microbial biomass C the biochar was mixed with field-moist soil, whereas for ergosterol and PLFA the soils were freeze-dried prior to biochar application. The incorporation of biochar into the soils just before microbial determinations and cell lysing through soil freeze-drying prevent microorganisms responding to biochar. Thus, the effects reported here are logically attributable to the chemical interference of biochar with extraction procedures, and not to microbiological responses. The caveat is that this experimental approach did not then allow examination of how duration of exposure affects chemical sorption of organic compounds onto biochar in soils before microbiological determinations.

Microbial biomass C was determined by fumigation–extraction (Vance et al., 1987). Ergosterol was determined by non-alkaline extraction in combination with sonication, followed by high performance liquid chromatography. An ergosterol spike of known concentration was used to correct for recovery (Ruzicka et al., 1995). The PLFA profile was determined according to Frostegård et al. (1993). The relative abundance (mol%) of the 40 most abundant fatty acids were subjected to principal component (PC) analysis to evaluate changes in microbial community composition. Statistical analysis of the resultant PC factor scores, and also the fumigation–extraction and ergosterol data, comprised of ANOVA and Fisher's LSD post-hoc test at the 0.05 level. All statistical analyses were performed with IBM SPSS Statistics, version 21 (Somers, NY).

Extractable C (before and after fumigation) and microbial biomass concentrations were significantly different between soil types (Table 1), with that of the clay soil having the greatest microbial biomass (Fig. 1). There was also a highly significant interaction between soil type and biochar production temperature on soil microbial biomass C (Table 1). In particular, biomass in the loamy sand and sandy loam soils was not significantly affected by biochar production temperature; however, the microbial biomass C content of the clay soil was significantly greater for the treatments with biochar produced at 300 °C than for those with biochar produced at 600 °C (Fig. 1c).

Extractable C from the non-fumigated clay soil decreased significantly after the addition of 50 g kg^{-1} of biochar produced at 300 °C, which resulted in a microbial biomass C significantly larger than that of the control (without biochar; Fig. 1c). This supports the observations of Durenkamp et al. (2010); indeed, they also observed a slight increase in a UK grassland soil with high microbial biomass after biochar addition at 28 g C kg^{-1}.

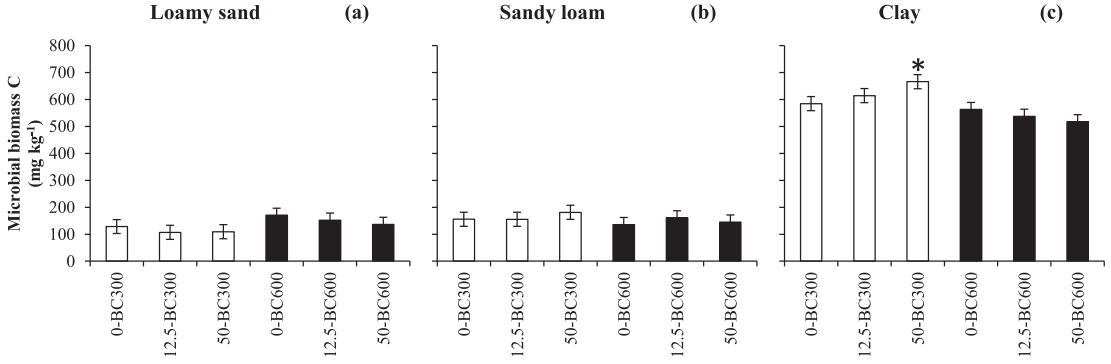

Fig. 1. Effect of the presence of 0 (control), 12.5, and 50 g kg^{-1} of biochar produced at 300 or 600 °C in loamy sand (a), sandy loam (b), and clay (c) soils on microbial biomass C content. Error bars indicate pooled standard error. Within each soil, asterisks indicate significant differences with respect to the corresponding control treatments (without biochar) according to Fisher's LSD test at the 0.05 level.