

Soil microbial responses over 2 years following biochar addition to a north temperate forest

Genevieve L. Noyce^{1,2} · Nathan Basiliko^{1,3} · Roberta Fulthorpe² · Tara E. Sackett⁴ · Sean C. Thomas⁴

Received: 16 October 2014 / Revised: 8 March 2015 / Accepted: 26 March 2015 / Published online: 9 April 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Soil microbial biomass, activity, and community composition were studied 1 and 2 years after biochar, phosphorus (P), and biochar+P additions to the soil of a north temperate, mixed-deciduous, P-limited forest in Central Ontario, Canada. Biochar was pyrolyzed on site from sugar maple and white spruce sawdust at ca. 400 °C, and P was added as triple superphosphate. Biochar additions of 5 t ha⁻¹ (approximately 0.4 to 1 kg P ha⁻¹) had minor effects on bacterial and fungal community composition, fungi/bacteria ratios, microbial biomass, and microbial C mineralization, with significant changes only being detected in the organic layer for additions of maple biochar. In contrast, additions of 200 kg P ha⁻¹ did alter soil chemical properties and reduced both microbial biomass and fungi/bacteria ratios. We conclude that biochar addition at 5 t ha⁻¹ is neither beneficial nor toxic to the soil microbes in a northern hardwood forest on acidic soils, suggesting that biochar amendments can be used to sequester C without adversely affecting the soil microbial community.

Keywords Biochar · Phosphorus · Bacteria · Fungi · qPCR · T-RFLP

Introduction

“Biochar,” or pyrolyzed biomass intended as a soil amendment, has received considerable recent attention as a means of enhancing C sequestration while improving productivity in managed ecosystems (Sohi et al. 2010; Lehmann et al. 2011). The effectiveness of biochar as a soil amendment is determined by its effect on the physical, chemical, and biological properties of the soil. These soil effects depend on several key features of the biochar particles, including surface area, pore-size distribution, water-holding capacity, and nutrient content, which in turn depend on the initial feedstock and the pyrolysis temperature (Lehmann et al. 2006; DeLuca et al. 2009; Singh et al. 2010; Mukherjee and Lal 2013; Ameloot et al. 2013).

Microbial responses to biochar depend strongly on soil type, vegetation, and other ecosystem attributes, as well as the properties of the biochar being added, making it difficult to synthesize disparate responses to biochar addition (Lehmann et al. 2011) and highlighting the need for studies that can characterize the microbial response to biochar with a variety of feedstocks, pyrolysis temperatures, soils, and climatic controls (Ameloot et al. 2013). To date, the majority of biochar-addition field studies have been conducted in tropical regions or on agricultural soils, which are not easily comparable to the temperate forests of Central Ontario or beyond. Tolerant hardwood forests on granitic parent material in Central Ontario commonly have moderately to highly acidic soils, requiring soil amendments to increase productivity. The most widely applied soil amendment in forests of this type is lime, which releases C to the atmosphere by chemical dissolution (West and McBride 2005), whereas biochar may instead

✉ Genevieve L. Noyce
genevieve.noyce@mail.utoronto.ca

¹ Department of Geography, University of Toronto, 100 St George Street, Toronto, ON M5S 3G3, Canada

² Department of Physical and Environmental Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON M1C 1A4, Canada

³ Department of Biology and the Vale Living with Lakes Centre, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON P3E 2C6, Canada

⁴ Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, Ontario M5S 3B3, Canada

sequester C. Fire suppression over the last century has reduced natural inputs of char to these forest ecosystems, but biochar may also emulate this natural disturbance (Wardle et al. 1998; DeLuca et al. 2006; Thomas 2013). In addition, recent studies indicate phosphorus (P) limitation of tree growth in this region, possibly as a response to high, persistent N deposition (Gradowski and Thomas 2006, 2008; Casson et al. 2012). Biochar can increase soil P availability, both by acting as a source and by reducing losses of P and other cations from the system (Sohi et al. 2010; Lehmann et al. 2011; Mukherjee and Lal 2013; Ameloot et al. 2013; Farrell et al. 2014). The effectiveness of biochar as a soil amendment is determined by its effects on the physical, chemical, and biological properties of the soil. These soil effects depend on several key features of the biochar particles, including surface area, pore-size distribution, water-holding capacity, and nutrient content, which in turn depend on the initial feedstock and the pyrolysis temperature (Lehmann et al. 2006; DeLuca et al. 2009; Singh et al. 2010; Mukherjee and Lal 2013; Ameloot et al. 2013).

While a few long-term (>12 months) field studies examining the effect of biochar on microbial biomass and growth rates, activity, and composition (Jones et al. 2012; Quilliam et al. 2012; Rousk et al. 2013; Domene et al. 2014) are beginning to emerge, none of them have been conducted in forested ecosystems. This study focuses on the effects of hardwood- and softwood-derived biochar and P addition on soil microbial community composition and functioning 1 and 2 years after addition in a temperate hardwood forest in Central Ontario. We build upon the work of Sackett et al. (2014), who characterized soil properties at this site up to 12 months after biochar addition and found that biochar increased availability of limiting plant nutrients. In addition, Mitchell et al. (2015) documented changes in soil microbial flora in a 24-week incubation experiment of these soils, most notably finding an increase in the ratio of bacteria to fungi and a decrease in the ratio of Gram-negative to Gram-positive bacteria with biochar addition. The field results presented here span 2 years post-addition, when we expected that surface-applied biochar would have pronounced effects on soil properties and, consequently, the biomass, activity, and composition of the soil microbial community. We hypothesized that, within the first few years after surface application, biochar would induce changes in soil chemistry that would in turn increase microbial biomass and activity and alter the composition of the soil microbial community, particularly by increasing the relative abundance and diversity of bacteria compared to fungi. We expected this shift in community composition to be mediated by pH increases and increased nutrient availability, both because fungi generally have lower biomass nutrient concentrations and internal pH and because mycorrhizal associations are favored in nutrient-limited soils.

Materials and methods

Site description and experimental design

Haliburton Forest and Wildlife Reserve is a 35,000-ha, privately owned forest in the Great Lakes–St. Lawrence region of Central Ontario, Canada (45.29° N, –78.64° W). The forest is situated on the Laurentian Shield and characterized by shallow sandy or sandy-loam dystric brunisols and humo-ferric podzols, with pH ranging from ca. 3.6 to 5.7 (Peng and Thomas 2006). The forest is classified as a tolerant hardwood forest; canopy dominant trees include sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*). Prior work has demonstrated P and/or Ca limitation of sugar maple at the site (Gradowski and Thomas 2006, 2008). The forest is managed by selection silviculture, which maintains uneven-aged stands. The experimental site was last logged during the winter of 2010 to 2011, with minimal forest floor impact.

A two-factor mixed design was applied to thirty 3×3-m plots in September 2011, just before leaf fall, with treatments allocated to ensure spatial interspersed and representation of a range of understory vegetation cover within each treatment (Sackett et al. 2014). Biochar treatments included controls (no biochar addition), 5 dry t ha⁻¹ of maple biochar, or 5 dry t ha⁻¹ of spruce biochar. The maple biochar treatment added 0.985 kg P ha⁻¹, and the spruce biochar treatment added 0.375 kg P ha⁻¹. In addition, plots were either left unfertilized or fertilized with triplesuperphosphate (Manchester Products, Canada) at 200 kg P ha⁻¹. All biochar was synthesized from sawdust using an 80-L batch pyrolyzer at Haliburton Forest, and maximum pyrolysis temperatures ranged from 350 to 450 °C. Biochar properties are listed in Table 1, and the analytical protocols are described by Sackett et al. (2014). The two biochars represent two predominant sources of feedstock regionally and also have varying amounts of extractable H⁺ and mineral nutrients, with maple biochar supplying twice as much Ca and P as the spruce biochar.

Soil collection and chemical analyses

Soil cores were collected in September 2012 and September 2013, 1 and 2 years after biochar addition, respectively. In 2012, three 5-cm-diameter soil cores of the top 5 cm of the mineral soil were collected from each plot. No organic material was included in these soil samples. In 2013, five 7-cm-diameter soil cores were collected from each plot and divided into the organic layer and the top 10 cm of the mineral soil. All soil cores were pooled per plot and frozen until analysis. Soil pH was measured in filtered extracts of 40 mL 0.5 M K₂SO₄:10 g soil. Soil moisture was determined gravimetrically. A subset of 2012 data (soil chemistry, microbial respiration, and microbial biomass) were already presented by

Table 1 Properties of experimental biochar (Sackett et al. 2014)

	Maple	Spruce
pH (H ₂ O)	7.7 (0.5)	6.5
% Carbon	77.3 (3.0)	77.9
% Nitrogen	0.1 (0.01)	0.01
% Sulfur	0.02 (0.00)	0.01
Ca (mg/kg)	6015 (944)	2419
K (mg/kg)	3443 (105)	1243
Mg (mg/kg)	619 (6)	285
P (mg/kg)	197 (16)	75

Maple values are mean (SD) for separate analyses of three batches used in pooled biochar. Spruce values are from single analysis of pooled biochar from multiple batches

Sackett et al. (2014), and thus only new data from 2013 are included here for these variables.

Microbial biomass and activity measurements

Microbial biomass C (MB-C) and N (MB-N) were determined using a modified chloroform (CHCl₃) fumigation-extraction method (Vance et al. 1987). Briefly, 10-g subsamples from each plot were fumigated with CHCl₃ stabilized with amylene in a vacuum desiccator. The fumigated samples and a replicate set of 10-g non-fumigated samples were each extracted with 40 mL 0.5 M K₂SO₄, filtered, and frozen until analysis. The dissolved organic C (DOC) and N (DON) concentrations of the extracts were measured on a TOC/TN analyzer (TOC-V_{CSH} with TNM-1, Shimadzu Corp., Japan). MB-C and MB-N were calculated as the difference in DOC and DON between the fumigated and non-fumigated samples without including an extraction efficiency constant.

Microbial respiration was measured in laboratory incubations. Subsamples (3 g wet weight) from each soil core were sealed in 30-mL serum vials and incubated at 20 °C for 24 h. One milliliter headspace samples were collected at 0, 4, 8, and 24 h, and CO₂ concentrations were measured using an infrared gas analyzer (S151, Qubit Systems, Canada) calibrated with a commercial CO₂ standard. Respiration was calculated as the linear increase in headspace CO₂ concentration over time and expressed per gram dry weight of soil. Nonlinear regressions (determined as data falling outside the 95 % confidence level) were discarded from the dataset.

DNA extraction, PCR, and T-RFLP analysis

DNA of the soil microbial community was extracted using PowerSoil DNA Isolation kits (MO BIO Laboratories, Inc., USA) following the manufacturer's instructions. To minimize extraction bias, soil samples were extracted in triplicate and

DNA was pooled prior to analysis. Bacterial 16S ribosomal RNA (rRNA) genes were amplified using fluorescently labeled forward primer Eu27f (5'-[6FAM] AGA GTT TGA TCM TGG CTC AG-3') and reverse primer Eu1492r (5'-ACG GYT ACC TTG TTA CGA CTT-3'). Fungal 18S rRNA genes were amplified using forward primer Fu817f (5'-TTA GCA TGG AAT AAT RRA ATA GGA-3') and fluorescently labeled reverse primer Fu1536r (5'-[6FAM] ATT GCA ATG CYC TAT CCC CA-3'). PCR amplification was conducted using either a Primus 96+ Thermocycler (MWG Biotech, Germany) or a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Canada), both with a program of 94 °C for 5 min, 30 cycles of 94 °C for 1 min, annealing component, and 72 °C for 2 min, followed by a 10-min extension at 72 °C. The annealing component was 1.5 min at 53 °C for the fungal primers and 1 min at 50 °C for the bacterial primers. PCR reactions for 2012 samples consisted of 2.5 µL 10× buffer, 4 µL MgCl₂, 0.5 µL dNTPs, 0.5 µL of each primer, 15.875 µL sterile water, and 1 µL of sample DNA. PCR reactions for 2013 samples consisted of 10 µL HotStarTaq Plus Master Mix (Qiagen, USA), 1 µL of each primer, 7 µL sterile water, and 1 µL of sample DNA. The PCR products from both sets were purified using GenElute PCR clean-up kits (Sigma-Aldrich, USA).

For terminal-restriction fragment length polymorphism (T-RFLP) analysis, 100 ng of purified DNA were digested with 10 U of restriction enzymes and 2 µL of buffer in 20-µL reactions at 37 °C for 3 h; 10 U MspI was used for bacteria, and 5 U each of AluI and MboI were used for fungi (New England BioLabs, USA). Terminal restriction fragments were measured relative to commercial standard fragments of known size (ROX 1000) at the University of Guelph Laboratory Services center (ON, Canada). Initial data filtering was performed according to Abdo et al. (2006). T-RF sizes were then rounded to the nearest integer and combined if their size was the same. Proportional abundances of each T-RF, within a given sample, were used for analysis. T-RFLP data for both years are presented here.

Quantitative PCR analysis

Relative abundances of fungi and bacteria in each soil sample were quantified using a modification of the technique described by Fierer et al. (2005). Quantitative PCR (qPCR) analysis was conducted using a 7300 Real-Time PCR System (Applied Biosystems, USA) with a program of 50 °C for 10 min, 95 °C for 15 min, 40 cycles of 95 °C for 1 min, and 53 °C for 30 s, followed by melting curve analysis. qPCR reactions consisted of 10 µL KAPA SYBR® FAST qPCR Master Mix (Kapa Biosystems, USA), 0.5 µL each of forward and reverse primers, 7 µL sterile water, and 15 ng soil community DNA. Bacterial 16S rRNA genes were amplified with Eub338 (5'-ACT CCT ACG GGA GGC AGC AG-3') and

Eub518 (5'-ATT ACC GCG GCT GCT GG-3'), and the fungal internal transcribed spacer (ITS) region was amplified with ITS1f (5'-TCC GTA GGT GAA CCT GCG G-3') and 5.8 s (5'-CGC TGC GTT CTT CAT CG-3') (Fierer et al. 2005). Each 96-well plate also contained reactions with ten-fold serial dilutions of pure bacterial and fungal DNA to verify the linearity of the relationship between threshold cycle (Ct) and DNA concentration. Sample Ct values were divided by the mean slope of the standard curves across all runs, to ensure that increases in fungal and bacterial abundance were weighted equally. Each sample was run twice, and the mean Ct value was used for analysis. The fungi/bacteria ratio was determined as the ratio of the adjusted ITS Ct to the adjusted 16S Ct. Ratios from both years were included in the analysis.

Statistical data analysis

R Project for Statistical Computing version 2.15.1 (R Core Team 2012) was used for all statistical analysis. Grubbs' test (function `grubbs.test` in package `{outliers}`; Komsta 2011) was used to identify outliers at $\alpha=0.005$, which were then removed from the dataset. No outliers were identified in the 2012 dataset. Four outliers were removed for 2013 (one moisture datapoint, one respiration datapoint, and two organic C datapoints), which is <1 % of the dataset. Multivariate analysis of variance (MANOVA; function `manova`) was conducted to determine the effects of soil layer, biochar feedstock, and P addition on 2013 soil pH, moisture, and organic C and N contents and the effects of soil layer, feedstock, P addition, pH, and moisture on 2013 microbial respiration, MB-C, MB-N, and the fungi/bacteria ratio. Due to the strong effect of soil layer in both analyses (soil properties $F_{8,41}=85.9$, $p<0.001$; microbial properties $F_{4,46}=62.3$, $p<0.001$), the MANOVAs were repeated for each layer individually. Post hoc analysis was conducted using univariate ANOVAs (function `aov`). *T* tests (function `t.test`) between means of soil and microbial properties were used to compare the effects of specific amendments (P, biochar, and biochar+P) relative to the control (no biochar, no P) plots in both years. T-RF data were square-root transformed to minimize the impact of dominant OTUs (Legendre and Legendre 1998). Community composition was analyzed with constrained correspondence analysis (CCA; function `cca` in package `{vegan}`; Oksanen et al. 2013) using Bray-Curtis dissimilarity, so that co-absences of a T-RF would not count as a similarity between two samples. Ordinations were constrained by biochar and phosphorus treatment. Differences in community composition between soil layers and treatments were evaluated using nonparametric multi-response permutation procedures (MRPP) with 5000 permutations per test. To assess the role of environmental factors, scores along the first two CCA axes for each plot were compared to vegetation cover, soil pH, soil moisture, soil OC, and soil ON using Pearson's correlation coefficient.

Results

Soil characteristics

There was generally no strong effect of biochar addition on soil characteristics (Table 2), and none of the biochar treatments significantly altered soil pH, organic C content, or organic N content, compared to the control plots, 2 years after addition (Fig. 1). However, soil moisture was significantly lower in the organic layer of the maple biochar plots compared to the control plots ($p=0.038$; Fig. 1b). There was also an overall effect of P addition in the organic layer (Table 2), which can be attributed to P fertilization significantly raising soil pH ($F_{4,21}=8.19$, $p<0.001$; Fig. 1a). In contrast, chemical properties of the mineral soil layer were not affected by P addition (Table 2). The organic layer was also significantly more acidic ($F_{1,51}=115.7$, $p<0.001$), drier ($F_{1,51}=659.0$, $p<0.001$), and contained more organic C ($F_{1,51}=181.1$, $p<0.001$) and organic N ($F_{1,51}=242.3$, $p<0.001$) than the mineral soil layer.

Microbial biomass, activity, and fungi/bacteria ratio

There was no significant effect of biochar addition on MB-C, MB-N, microbial activity, or the fungi/bacteria ratio in either soil layer in 2013 (Table 3). In the organic layer, only pH had a significant overall effect (Table 3), which was caused by a strong effect of soil pH on the fungi/bacteria ratio ($F_{1,22}=5.07$, $p=0.035$). However, the addition of maple biochar, both by itself and in conjunction with P, significantly lowered rates of microbial respiration in the organic layer (maple: $p=0.041$; maple+P: $p=0.030$; Fig. 1e). There was no effect of maple biochar on microbial respiration in the mineral soil layer, but pH did have a significant effect ($F_{1,22}=6.40$, $p=0.019$).

P addition did not have an overall microbial effect in the organic layer (Table 3), though MB-C was significantly lower in plots with added P ($F_{1,22}=5.92$, $p=0.024$; Fig. 1f). There was a significant overall effect in the mineral soil layer (Table 3), due to lower MB-C ($F_{1,22}=19.9$, $p<0.001$) and MB-N ($F_{1,22}=23.6$, $p<0.001$) after P addition. In particular, both P and maple+P treatments significantly reduced MB-N (P: $p=0.034$; maple+P: $p=0.035$; Fig. 1g). In 2013, fungi/bacteria ratios were significantly lower in the organic layer of the P and maple treatments compared to the controls (P: $p=0.012$, maple: $p=0.032$; Fig. 2). There were no significant treatment effects in the mineral soil layer in either year (data not shown).

The 2013 organic and mineral soil layers were also significantly different from each other in every microbial analysis (Fig 1). The organic layer had higher rates of microbial respiration ($F_{1,49}=192.8$, $p<0.001$), higher MB-C ($F_{1,49}=68.8$, $p<0.001$) and MB-N ($F_{1,49}=146.8$, $p<0.001$), and a lower fungi/bacteria ratio ($F_{1,49}=18.2$, $p<0.001$).

Table 2 MANOVA results for variables affecting soil pH, moisture, organic C, and organic N in 2013

	Pillai's trace	F	p
Organic			
Biochar feedstock	0.426	1.49	0.189
Phosphorus addition	0.586	7.43	<0.001
Mineral			
Biochar feedstock	0.175	0.527	0.830
Phosphorus addition	0.087	0.501	0.736

Bold text indicators significant factors ($p < 0.05$)

Microbial community composition

In 2012, biochar type had a marginally significant effect on the overall microbial community composition ($p = 0.093$) as well as the fungal community composition ($p = 0.064$; Fig. 3d) but no effect on the bacterial community (Fig. 3a). There was also no effect of P addition on microbial community composition (Fig. 3a, d).

In 2013, there were no significant treatment effects on community composition across the entire dataset, but there were strong differences in microbial community composition between the organic and mineral soil layers ($p < 0.001$). When the soils were examined separately, there were still no biochar or P treatment effects in the organic layer (Fig. 3b, e), but there was a significant effect of biochar type on overall community

Table 3 MANOVA results for variables affecting microbial biomass, respiration, and fungi/bacteria ratio in 2013

	Pillai's trace	F	p
Organic			
Biochar feedstock	0.342	1.03	0.428
Phosphorus addition	0.299	2.03	0.131
Soil pH	0.452	3.92	0.017
Soil moisture	0.177	1.02	0.422
Mineral			
Biochar feedstock	0.243	0.693	0.695
Phosphorus addition	0.541	5.59	0.004
Soil pH	0.322	2.25	0.101
Soil moisture	0.079	0.409	0.800

Bold text indicates significant factors ($p < 0.05$)

composition in the mineral soil ($p = 0.049$). This was driven by a significant effect of biochar addition on fungal community composition ($p = 0.046$; Fig. 3f) and a marginally significant effect of biochar type on bacterial community composition ($p = 0.051$; Fig. 3c). Biochar type effects were not significant for the fungal community. Phosphorus addition had no effect on the community composition in the mineral soil layer.

The CCA axes were only correlated with select environmental variables. Mineral soil bacterial community composition was correlated with soil pH in 2012 ($r = 0.31$, $p = 0.009$)

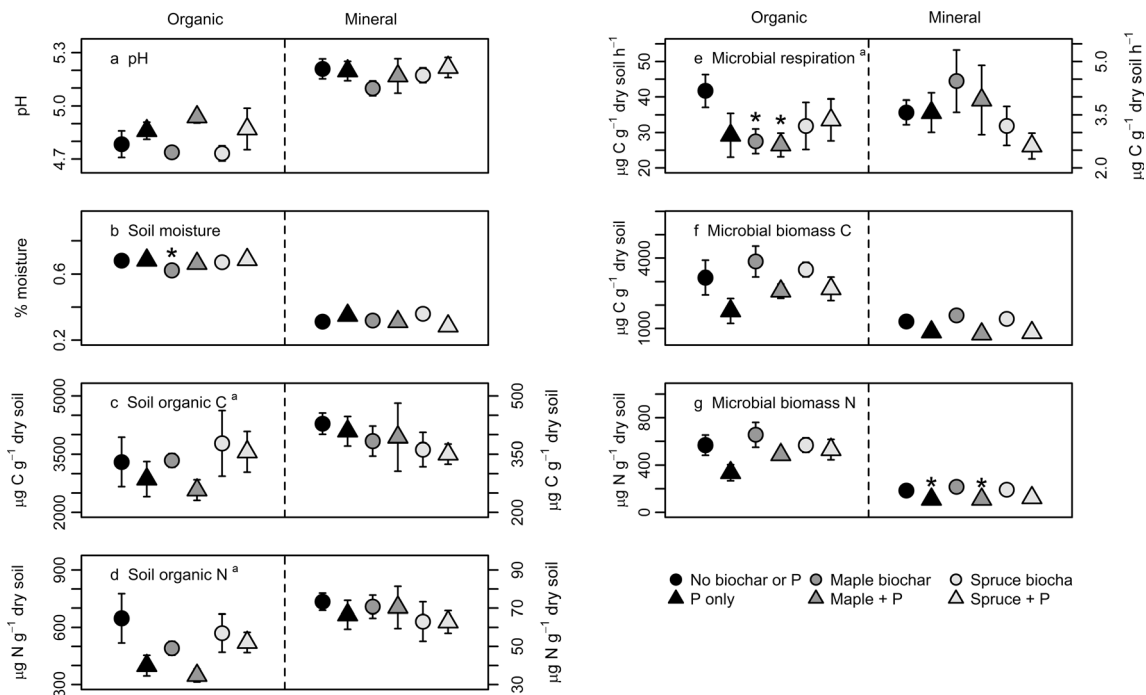


Fig. 1 Means ($n = 5$) per treatment and soil layer of **a** soil pH, **b** soil moisture, **c** soil organic C, **d** soil organic N, **e** microbial respiration, **f** MB-C, and **g** MB-N in 2013 soil samples. Colors indicate biochar treatment: no biochar (black), maple biochar (dark gray), or spruce biochar (light gray). Shapes indicate P treatment: without added P (circles) or

with added P (triangles). Error bars represent one SE. *a* indicates scale for mineral data is one order of magnitude smaller than scale for organic data. Asterisk indicates treatment mean is significantly ($p < 0.05$) different from control (no biochar, no P) mean

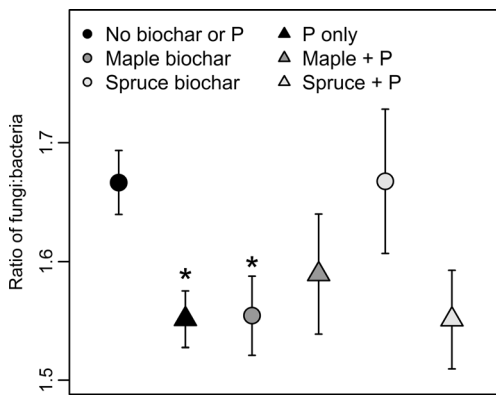


Fig. 2 Means ($n=5$) per treatment of fungi/bacteria ratio in 2013 organic layer samples. Colors indicate biochar treatment: no biochar (black), maple biochar (dark gray), or spruce biochar (light gray). Shapes indicate P treatment: without added P (circles) or with added P (triangles). Error bars represent one SE. Asterisk indicates treatment mean is significantly ($p<0.05$) different from control (no biochar, no P) mean

but not with any of the measured soil chemistry properties in either soil layer in 2013 (Fig. 3a–c). Mineral soil fungal community composition was significantly correlated with soil moisture in 2013 ($r=0.31, p=0.011$) but not with any variable

in either the organic layer in 2013 or the mineral soil layer in 2012 (Fig. 3d–f).

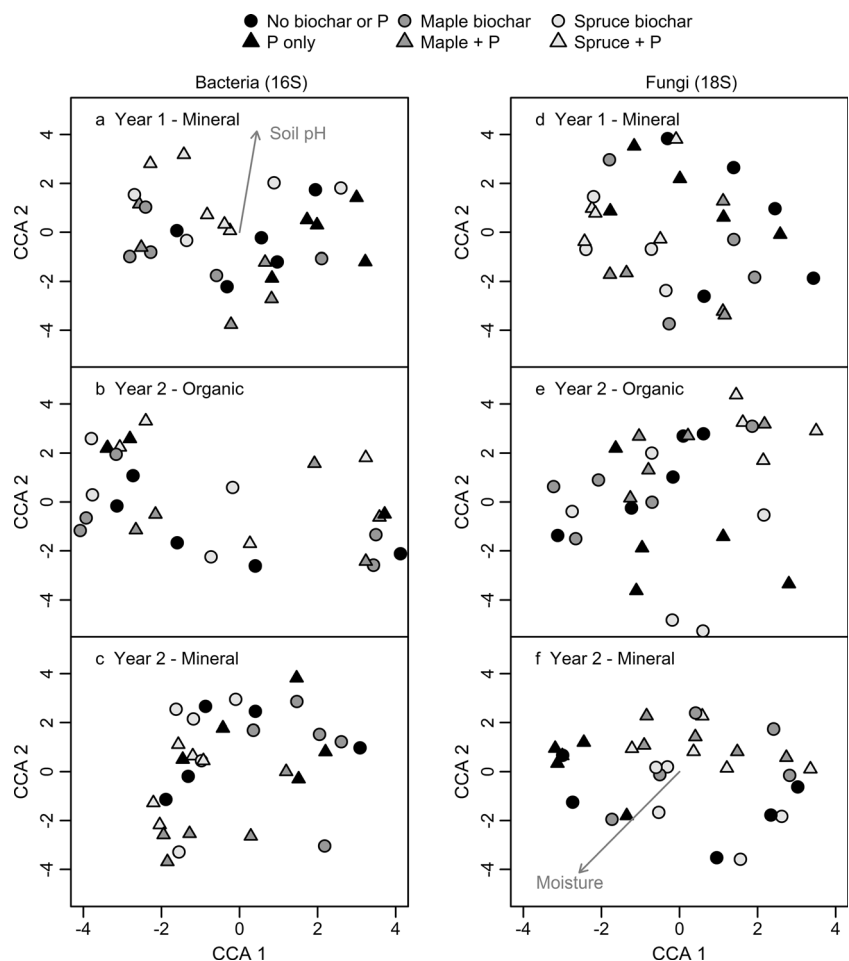
Discussion

We obtained primarily null results in the present study, with biochar additions resulting in no statistically significant effects on most of the soil chemical properties or on microbial community composition and function. However, the positive results found suggest important feedstock-specific effects: maple biochar addition caused a detectable decrease in organic soil layer moisture content, along with a reduction in microbial respiration and a decreased fungi/bacteria ratio, whereas spruce biochar had no effect. Biochar additions also resulted in small but detectable shifts in fungal and bacterial community composition in the mineral soil after 2 years.

Soil chemistry

The lack of changes in soil properties in the mineral soil layer in 2012 (Sackett et al. 2014) was potentially because the

Fig. 3 a–f CCA ordination plots based on 16S and 18S T-RFs in soil samples from both a–c 2012 and d–f 2013 with biochar and P treatment as constraints, separated by soil layer. Colors indicate biochar treatment: no biochar (black), maple biochar (dark gray), or spruce biochar (light gray). Shapes indicate P treatment: without added P (circles) or with added P (triangles). Arrows represent environmental variables that were significantly correlated with the CCA axes at $\alpha=0.05$



surface-applied biochar had yet to be incorporated into the mineral soil. By 2013, the biochar particles were clearly mixed into the organic soil layer and consequently biochar-induced changes in soil chemistry were expected. The lack of pH effect was particularly surprising, given that many previous studies found that biochar applications increased soil pH, especially in acidic soils (Biederman and Harpole 2013). This null effect was likely due to the buffering capacity of the soil organic layer, such that adding biochar at more than 5 t ha^{-1} would effect a change in soil pH. In an agricultural study, adding biochar at 30 t ha^{-1} significantly increased soil pH, while additions of 3 to 12 t ha^{-1} did not (Domene et al. 2014). Long-term studies in agricultural soils also suggest that pH responses to biochar may be transient (Jones et al. 2012; Quilliam et al. 2012), which may explain the lack of 2013 pH effects. However, we did find substantial effects of biochar additions on available P, Ca, and Mg in mineral soils within the first year following addition (Sackett et al. 2014).

One explanation for the limited chemical effects could be that the biochar particles migrated from the plots after addition, given the shallow, medium- to coarse-textured soils characteristic of this forest, but we believe this is unlikely to have occurred, especially because the biochar was added immediately prior to autumn leaf fall to minimize erosion losses. While some mobile species from the biochar likely leached after addition (Sackett et al. 2014), char particles were still visually present throughout the soil of treated areas in 2013. In addition, when biochar particles of varying sizes were deployed in Haliburton Forest, the char lost approximately 5–10 % of its mass initially but then remained stable for at least 4 years (C. Winsborough, personal communication).

Biochar effects on the soil microbial community

Microbial biomass

In the present field study, there was no effect of biochar addition on microbial biomass in either 2012 (Sackett et al. 2014) or 2013, despite increased nutrient availability in 2012. Even though a recent meta-analysis found that MB-C typically increases after biochar addition (Biederman and Harpole 2013), other medium-term field studies of biochar addition have similarly found a negligible change in microbial biomass several years after the biochar was added, suggesting that these effects are generally short-lived (Jones et al. 2012; Rousk et al. 2013). Observed increases in microbial biomass are commonly attributed to greater C and nutrient availability (Lehmann et al. 2011; Biederman and Harpole 2013), either supplied directly or via increased plant root activity, so these results may indicate that by 12 months after biochar addition, there was no labile C remaining for use by the microbial community (Anders et al. 2013).

The null effect on microbial biomass may also be a function of the consistency of other soil properties across treatments. Domene et al. (2014) found that their observed increase in MB-C was mainly due to increased soil moisture after biochar addition, whereas in this study, spruce biochar had no effect on soil moisture and maple biochar decreased soil moisture by 8.5 %. Alternatively, the negligible effect may also be due to the low biochar addition rate; Domene et al. (2014) only observed an increase in MB-C after adding biochar at 30 t ha^{-1} . Incubation experiments have also only found biochar to affect microbial biomass at high addition rates (Kolb et al. 2009).

Microbial activity

Biochar addition was expected to increase microbial activity, causing a release of C (Luo et al. 2011), as was documented in 24-week incubations of the same soil by Mitchell et al. (2015). This effect is thought to be due to either the mineralization of labile C in the biochar particles or the stimulation of microbial activity through additions of labile nutrients (Mukherjee and Lal 2013; Ameloot et al. 2013), though these effects can be short-lived (Steinbeiss et al. 2009). In this study, microbial respiration in the organic layer of the maple biochar plots was 42 % lower than in the control plots even though there was no change in microbial biomass. This may have been an example of “negative priming,” in which biochar addition decreases the overall respiration rate of native soil organic matter possibly due to physical protection of soil particles from microbial degradation (Zimmerman et al. 2011; Jones et al. 2011; Ameloot et al. 2013; Prayogo et al. 2014) or the ability of biochar to chemisorb CO_2 , leading to underestimated microbial C mineralization rates (Ameloot et al. 2013). Our results also indicate a strong effect of feedstock on biochar-microbial interactions in the soil, because there were no changes in microbial respiration in the spruce biochar plots. Similar differences between biochars have also been seen in short-term incubation studies of forest soils: Khodadad et al. (2011) found that microbial respiration rates depended on the pyrolysis temperature and feedstock of the added biochar. Total soil respiration rates were not measured in 2013, but Sackett et al. (2014) found that even when microbial respiration rates in 2012 were significantly affected by biochar addition, field measurements of soil CO_2 efflux did not change. This minimal effect of biochar on total soil respiration has also been seen in agricultural soils (Jones et al. 2012). In sum, our results indicate that biochar addition at 5 t ha^{-1} sequesters C in temperate forest soils without increasing respiration of native organic matter, under field conditions.

Microbial community composition

Surprisingly, the fungi/bacteria ratios in these soils were generally not affected by biochar addition in either 2012 or 2013. This is in contrast to other experiments, in which lower fungi/bacteria ratios have been observed 1 to 2 years after biochar addition (Jin 2010; Chen et al. 2013), and specifically contradicts results from a short-term incubation study using the same soil (Mitchell et al. 2015). However, Mitchell et al. (2015) also found that the main effects occurred at biochar addition rates of 10 to 20 t ha⁻¹, whereas the control and 5 t ha⁻¹ soils had a similar microbial community. Our null result is likely linked to the fact that there was no overall effect of adding biochar at 5 t ha⁻¹ on soil pH, as discussed previously. Changes in microbial community composition after biochar addition are often correlated with pH (Rousk et al. 2013; Anders et al. 2013), and in the present study, soil pH was the main control on the fungi/bacteria ratio in the organic layer and pH was also correlated with bacterial community composition in 2012. There was also a significant decrease in the fungi/bacteria ratio in the organic layer of the maple biochar plots, which may have been linked to pH changes. The values reported here reflect the pH of the bulk soil, which may be different from the pH in the immediate vicinity of the biochar particles (Lehmann et al. 2011). If pH increased in the soil zone near a biochar particle, increased bacterial growth and reduced fungal growth may occur (Rousk et al. 2009, 2010), which could explain the observed lower fungi/bacteria ratio. In the present study, this would only have occurred in the organic layer where the biochar particles were incorporated into the soil. In addition, the maple biochar was more alkaline than the spruce biochar, which may explain why there was no observed effect of spruce biochar addition. However, changes in community composition after biochar addition are not always attributable to changes in soil pH (Prayogo et al. 2014).

Biochar-induced changes in soil physical and chemical properties were expected to alter the relative abundance of common soil bacterial groups, as has been seen in previous studies shortly after biochar addition (Steinbeiss et al. 2009; Grossman et al. 2010; Khodadad et al. 2011; Kolton et al. 2011; Chen et al. 2013; Anders et al. 2013; Gomez et al. 2014; Prayogo et al. 2014; Song et al. 2014; Mitchell et al. 2015). Instead, 2 years after biochar was applied to the surface of the forest floor, there were no detectable differences in the composition of either the bacterial or fungal communities in the organic layer. There were, however, detectable treatment responses in the mineral soil communities in both years, although the distribution of taxa was still very similar. Extracting microbial DNA from biochar-amended soils can be challenging because DNA can be sorbed to the negatively charged biochar particles (Lehmann et al. 2011). However, this is mainly a problem at high addition rates, and Jin (2010) found that amending the DNA

extraction method did not alter T-RFLP results, so that is unlikely to be of concern in the present study. Instead, our results imply that 5 t ha⁻¹ biochar additions to this site have only minor effects on the native microbial community composition.

During pyrolysis, most of the feedstock C is incorporated into recalcitrant condensed aromatic rings, but some residues of volatile organic compounds condense on the outside of the biochar particles (Ameloot et al. 2013). After addition to soil, these compounds are rapidly mineralized, whereas mineralization of the aromatic structures is minimal, meaning that there is generally an initial pulse in C and mineral nutrient release after biochar addition, but over time, the differences between control and biochar-amended soils decline (Jones et al. 2012; Quilliam et al. 2012; Ameloot et al. 2013; Rousk et al. 2013). Soil nutrient availability was not measured in 2013, but Sackett et al. (2014) found that most nutrient effects observed within 2 to 6 weeks after biochar addition were no longer present by the end of the first year (other than increased Ca²⁺ levels), suggesting that many nutrient effects at this site were transient. Similarly, the resiliency of the microbial community should likewise result in only transient effects of biochar additions (Quilliam et al. 2012; Rousk et al. 2013). In this study, we found effects on the mineral soil microbial community composition, but not in the organic soil layer, in the second year after biochar addition.

The most important difference with prior studies is likely that biochar was added as surface dressing that was then incorporated into the soil by natural mixing with decomposing litterfall. Mitchell et al. (2015) observed strong biochar effects on the microbial community composition in short-term incubations of these soils, but that experiment only included mineral soil, so there were no interactions with litter or roots, and the biochar was thoroughly mixed into the soil rather than being applied on the surface. We speculate that the forest soil organic layer may have a particularly high buffering capacity, and also a more resilient microbial community than that found in agricultural systems, such that changes in the soil microbial community composition and functioning are delayed and occur mainly in the mineral soil.

Phosphorus effects on the soil microbial community

Phosphorus-fertilization effects on microbial biomass were most likely due to elevated soil pH and increased nutrient availability, though feedbacks involving plant root responses are also possible. The significant increase in pH was presumably from the calcium component of the added triple superphosphate and likely contributed to the significantly lower fungi/bacteria ratio. P-fertilized plots also had significantly lower microbial biomass, which may be due to a decline in mycorrhizae, with plants now having easier access to the previously limiting nutrient (van der Heijden et al. 2008; van der

Heijden and Horton 2009; Blanes et al. 2012). The lower fungi/bacteria ratio may also indicate a decline in mycorrhizal fungi in these plots, though the reduction in microbial biomass could also have occurred in response to increased competition for nutrients with plant roots, once P limitation was alleviated (Blanes et al. 2012).

Despite the changes in soil pH and fungi/bacteria ratio, there was no effect of P treatment on microbial community composition, in contrast to prior studies (Wakelin et al. 2009; Kluber et al. 2012). It is possible that either P addition did not alter the relative abundances of the observed taxa or that the resolution of the T-RFLP community fingerprinting approach was not sufficient to detect changes in individual functional groups. However, T-RFLP is a widely used method that has routinely shown microbial community changes in other soils post-fertilization (e.g., Bissett et al. 2013; Högberg et al. 2014). Even though P is a limiting nutrient at this site, there was also no discernible effect of P addition on microbial respiration. This is in contrast to a prior study conducted in Haliburton Forest, in which P fertilization substantially reduced microbial respiration (Peng and Thomas 2010). However, in that case, P and K were added to the soil for two consecutive years prior to measuring respiration, and the additional nutrient loading is thus more likely to have reduced P limitation and thus have had a stronger effect on microbial community functioning. Direct P and Ca additions may mimic, in an exaggerated manner, soil responses to biochar additions at much high dosages than those used in this experiment. The lack of microbial community responses to P additions thus suggests that higher biochar dosages would similarly result in little or no response of the soil microbial community in this system.

Conclusions

The effects of biochar on forest soil microbial community composition and functioning in the first 2 years after addition were limited. Neither biochar type altered microbial biomass in either soil layer or community composition in the organic layer, implying that biochar addition at 5 t ha⁻¹ is neither beneficial nor toxic to the soil microbes at this site. Although minor changes in the mineral soil layer community composition were observed, there was still substantial overlap in microbial taxa between treatments. Similarly, P fertilization also had no detectable effect on microbial community composition. These results provide a further illustration that biochar does not have universal effects and caution that the large effects on microbial communities that are observed in short-term incubation studies (Mitchell et al. 2015) do not necessarily provide a good indicator of longer-term impacts in the field. While maple biochar altered microbial properties, there was no corresponding effect of spruce biochar addition,

indicating the strong variability of effects depending on the biochar being added. Ultimately, we have demonstrated that biochar does not negatively affect forest soil microbial communities. Prior to this experiment, no field studies had assessed the ecosystem effects of biochar in a north temperate forest beyond the initial 12 months after biochar addition. This work is necessary in forested ecosystems where long-term effects are more important than in agricultural systems with yearly crop turnover. These results, combined with the earlier work of Sackett et al. (2014) demonstrating short-lived effects on soil chemical properties and greenhouse gas exchange, imply that biochar additions at low to moderate dosages are unlikely to have negative long-term effects on ecosystem functioning.

Acknowledgments Funding for this project came from a NSERC Strategic Projects Grant to S. Thomas, N. Basiliko, and others and graduate fellowship support from the University of Toronto to G. Noyce. Haliburton Forest and Wildlife Reserve graciously allowed site access and provided subsidized field lodging as well as personnel, equipment, and feedstock for biochar synthesis. We thank Kira Borden, Owen Brokenshire, Malcom Cockwell, Matt Garmon, Maciej Jamrozic, Kaitlyn Loukes, Leo Rocca, Jonathan Schurman, and Carolyn Winsborough for the assistance with setting up the plots and pyrolyzing the biochar and Chihiro Ikeda for assistance with 2012 lab work.

References

- Abdo Z, Schüette UME, Bent SJ, Williams CJ, Forney LJ, Joyce P (2006) Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. *Environ Microbiol* 8:929–938. doi:10.1111/j.1462-2920.2005.00959.x
- Ameloot N, Graber ER, Verheijen FGA, De Neve S (2013) Interactions between biochar stability and soil organisms: review and research needs. *Eur J Soil Sci* 64:379–390. doi:10.1111/ejss.12064
- Anders E, Watzinger A, Rempt F, Kitzler B, Wimmer B, Zehetner F, Stahr K, Zechmeister-Boltenstern S, Soja G (2013) Biochar affects the structure rather than the total biomass of microbial communities in temperate soils. *Agric Food Sci* 22:404–423
- Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *GCB Bioenergy* 5:202–214. doi:10.1111/gcbb.12037
- Bissett A, Richardson AE, Baker G, Kirkegaard J, Thrall PH (2013) Bacterial community response to tillage and nutrient additions in a long-term wheat cropping experiment. *Soil Biol Biochem* 58:281–292. doi:10.1016/j.soilbio.2012.12.002
- Blanes MC, Emmett BA, Viñepla B, Carreira JA (2012) Alleviation of P limitation makes tree roots competitive for N against microbes in a N-saturated conifer forest: a test through P fertilization and ¹⁵N labelling. *Soil Biol Biochem* 48:51–59. doi:10.1016/j.soilbio.2012.01.012
- Casson NJ, Eimers MC, Watmough SA (2012) An assessment of the nutrient status of sugar maple in Ontario: indications of phosphorus limitation. *Environ Monit Assess* 184:5917–5927
- Chen J, Liu X, Zheng J, Zhang B, Lu H, Chi Z, Pan G, Li L, Zheng J, Zhang X, Wang J, Yu X (2013) Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Appl Soil Ecol* 71:33–44. doi:10.1016/j.apsoil.2013.05.003

- DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. *Soil Sci Soc Am J* 70:448–453. doi:10.2136/sssaj2005.0096
- DeLuca TH, MacKenzie MD, Gundale MJ (2009) Chapter 14: Biochar effects on soil nutrient transformations. In: Lehmann J, Joseph S (eds) *Biochar for environmental management*. Earthscan, London, pp 251–270
- Domene X, Mattana S, Hanley K, Enders A, Lehmann J (2014) Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn. *Soil Biol Biochem* 72:152–162. doi:10.1016/j.soilbio.2014.01.035
- Farrell M, Macdonald LM, Butler G, Chirino-Valle I, Condon L (2014) Biochar and fertiliser applications influence phosphorus fractionation and wheat yield. *Biol Fertil Soils* 50:169–178. doi:10.1007/s00374-013-0845-z
- Fierer N, Jackson JA, Vilgalys R, Jackson RB (2005) Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl Environ Microbiol* 71:4117–4120. doi:10.1128/AEM.71.7.4117-4120.2005
- Gomez JD, Deneff K, Stewart CE, Zheng J, Cotrufo MF (2014) Biochar addition rate influences soil microbial abundance and activity in temperate soils. *Eur J Soil Sci* 65:28–39. doi:10.1111/ejss.12097
- Gradowski T, Thomas SC (2006) Phosphorus limitation of sugar maple growth in central Ontario. *For Ecol Manag* 226:104–109. doi:10.1016/j.foreco.2005.12.062
- Gradowski T, Thomas SC (2008) Responses of *Acer saccharum* canopy trees and saplings to P, K and lime additions under high N deposition. *Tree Physiol* 28:173–185. doi:10.1093/treephys/28.2.173
- Grossman JM, O'Neill BE, Tsai SM, Liang B, Neves E, Lehmann J, Thies JE (2010) Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. *Microb Ecol* 60:192–205. doi:10.1007/s00248-010-9689-3
- Högberg MN, Yarwood SA, Myrold DD (2014) Fungal but not bacterial soil communities recover after termination of decadal nitrogen additions to boreal forest. *Soil Biol Biochem* 72:35–43. doi:10.1016/j.soilbio.2014.01.014
- Jin H (2010) Characterization of microbial life colonizing biochar and biochar-amended soils. PhD Dissertation, Cornell University
- Jones DL, Murphy DV, Khalid M, Ahmad E, Edwards-Jones G, DeLuca TH (2011) Short-term biochar-induced increase in soil CO₂ release is both biotically and abiotically mediated. *Soil Biol Biochem* 43:1723–1731. doi:10.1016/j.soilbio.2011.04.018
- Jones DL, Rousk J, Edwards-Jones G, DeLuca TH, Murphy DV (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biol Biochem* 45:113–124. doi:10.1016/j.soilbio.2011.10.012
- Khodadad CLM, Zimmerman AR, Green SJ, Uthandi S, Foster JS (2011) Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol Biochem* 43:385–392. doi:10.1016/j.soilbio.2010.11.005
- Kluber LA, Carrino-Kyker SR, Coyle KP, DeForest JL, Hewins CR, Shaw AN, Smemo KA, Burke DJ (2012) Mycorrhizal response to experimental pH and P manipulation in acidic hardwood forests. *PLoS ONE* 7:e48946. doi:10.1371/journal.pone.0048946
- Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of charcoal quantity on microbial biomass and activity in temperate soils. *Soil Sci Soc Am J* 73:1173. doi:10.2136/sssaj2008.0232
- Kolton M, Harel YM, Pasternak Z, Graber ER, Elad Y, Cytryn E (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Appl Environ Microbiol* 77:4924–4930. doi:10.1128/AEM.00148-11
- Komsta L (2011) *Outliers: test for outliers*
- Legendre P, Legendre L (1998) *Numerical ecology*, 2nd edn. Elsevier
- Lehmann J, Gaunt J, Rondon M (2006) Biochar sequestration in terrestrial ecosystems: a review. *Mitig Adapt Strateg Glob Chang* 11:395–419
- Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota: a review. *Soil Biol Biochem* 43:1812–1836. doi:10.1016/j.soilbio.2011.04.022
- Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes PC (2011) Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biol Biochem* 43:2304–2314. doi:10.1016/j.soilbio.2011.07.020
- Mitchell PJ, Simpson AJ, Soong R, Simpson MJ (2015) Shifts in microbial community and water-extractable organic matter composition with biochar amendment in a temperate forest soil. *Soil Biol Biochem* 81:244–254. doi:10.1016/j.soilbio.2014.11.017
- Mukherjee A, Lal R (2013) Biochar impacts on soil physical properties and greenhouse gas emissions. *Agronomy* 3:313–339. doi:10.3390/agronomy3020313
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2013) *vegan: community ecology package*
- Peng Y, Thomas SC (2006) Soil CO₂ efflux in uneven-aged managed forests: temporal patterns following harvest and effects of edaphic heterogeneity. *Plant Soil* 289:253–264. doi:10.1007/s11104-006-9133-0
- Peng Y, Thomas SC (2010) Influence of non-nitrogenous soil amendments on soil CO₂ efflux and fine root production in an N-saturated northern hardwood forest. *Ecosystems* 13:1145–1156. doi:10.1007/s10021-010-9379-5
- Prayogo C, Jones JE, Baeyens J, Bending GD (2014) Impact of biochar on mineralisation of C and N from soil and willow litter and its relationship with microbial community biomass and structure. *Biol Fertil Soils* 50:695–702. doi:10.1007/s00374-013-0884-5
- Quilliam RS, Marsden KA, Gertler C, Rousk J, DeLuca TH, Jones DL (2012) Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agric Ecosyst Environ* 158:192–199. doi:10.1016/j.agee.2012.06.011
- Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl Environ Microbiol* 75:1589–1596. doi:10.1128/AEM.02775-08
- Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351. doi:10.1038/ismej.2010.58
- Rousk J, Dempster DN, Jones DL (2013) Transient biochar effects on decomposer microbial growth rates: evidence from two agricultural case-studies. *Eur J Soil Sci* 64:770–776. doi:10.1111/ejss.12103
- R Core Team (2012) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria
- Sackett TE, Basiliko N, Noyce GL, Winsborough C, Schurman J, Ikeda C, Thomas SC (2014) Soil and greenhouse gas responses to biochar addition in a temperate hardwood forest. *GCB Bioenergy*. doi:10.1111/gcbb.12211
- Singh B, Singh BP, Cowie AL (2010) Characterisation and evaluation of biochars for their application as a soil amendment. *Soil Res* 48:516–525
- Sohi SP, Krull E, Lopez-Capel E, Bol R (2010) A review of biochar and its use and function in soil. In: Sparks DL (ed) *Advances in agronomy*. Academic Press, London, pp 47–82
- Song Y, Zhang X, Ma B, Chang SX, Gong J (2014) Biochar addition affected the dynamics of ammonia oxidizers and nitrification in microcosms of a coastal alkaline soil. *Biol Fertil Soils* 50:321–332. doi:10.1007/s00374-013-0857-8

- Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol Biochem* 41:1301–1310. doi:[10.1016/j.soilbio.2009.03.016](https://doi.org/10.1016/j.soilbio.2009.03.016)
- Thomas SC (2013) Biochar and its potential in Canadian forestry. *Silvic Mag* 2013:4–6
- Van der Heijden MGA, Horton TR (2009) Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *J Ecol* 97:1139–1150. doi:[10.1111/j.1365-2745.2009.01570.x](https://doi.org/10.1111/j.1365-2745.2009.01570.x)
- Van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296–310. doi:[10.1111/j.1461-0248.2007.01139.x](https://doi.org/10.1111/j.1461-0248.2007.01139.x)
- Vance E, Brookes P, Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703–707
- Wakelin SA, Gregg AL, Simpson RJ, Li GD, Riley IT, McKay AC (2009) Pasture management clearly affects soil microbial community structure and N-cycling bacteria. *Pedobiologia* 52:237–251. doi:[10.1016/j.pedobi.2008.10.001](https://doi.org/10.1016/j.pedobi.2008.10.001)
- Wardle DA, Zackrisson O, Nilsson M-C (1998) The charcoal effect in boreal forests: mechanisms and ecological consequences. *Oecologia* 115:419–426. doi:[10.1007/s004420050536](https://doi.org/10.1007/s004420050536)
- West TO, McBride AC (2005) The contribution of agricultural lime to carbon dioxide emissions in the United States: dissolution, transport, and net emissions. *Agric Ecosyst Environ* 108:145–154. doi:[10.1016/j.agee.2005.01.002](https://doi.org/10.1016/j.agee.2005.01.002)
- Zimmerman AR, Gao B, Ahn M-Y (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol Biochem* 43:1169–1179. doi:[10.1016/j.soilbio.2011.02.005](https://doi.org/10.1016/j.soilbio.2011.02.005)