Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition

Perry J. Mitchell, André J. Simpson, Ronald Soong, Jonathan S. Schurman Sean C. Thomas, Myrna J. Simpson

Version Post-print/accepted manuscript

Citation Mitchell, P.J., Simpson, A.J., Soong, R. et al. *Biogeochemistry* (2016)
(published version) 130: 227. <https://doi.org/10.1007/s10533-016-0254-0>

Publisher's statement This is a post-peer-review, pre-copyedit version of an article published in *Biogeochemistry*. The final authenticated version is available online at: <https://doi.org/10.1007/s10533-016-0254-0>.

How to cite TSpace items

Always cite the published version, so the author(s) will receive recognition through services that track citation counts, e.g. Scopus. If you need to cite the page number of the **author manuscript from TSpace** because you cannot access the published version, then cite the TSpace version **in addition to** the published version using the permanent URI (handle) found on the record page.

This article was made openly accessible by U of T Faculty.
Please [tell us](#) how this access benefits you. Your story matters.

Biochar amendment and phosphorus fertilization altered forest soil microbial community and native soil organic matter molecular composition

Perry J. Mitchell^{a,b}, André J. Simpson^{a,b}, Ronald Soong^b, Jonathan S. Schurman^c, Sean C. Thomas^c, Myrna J. Simpson^{a,b*}

^a Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON, M5S 3H6, Canada; ^b Environmental NMR Centre and Department of Physical and Environmental Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON, M1C 1A4, Canada; ^c Faculty of Forestry, University of Toronto, 33 Willcocks Street, Toronto, ON, M5S 3B3, Canada; * Corresponding author: Tel.: +1 (416) 287-7234, Fax: +1 (416) 287-7279, E-mail address: myrna.simpson@utoronto.ca (M.J. Simpson).

Abstract

Fertilizer application to nutrient-deficient forest soils may alleviate soil nutrient limitations, but long-term application may be cost-prohibitive and logistically challenging to apply to large areas. Biochar has been proposed as a soil amendment to increase soil carbon storage and may emulate fertilizer application by increasing soil nutrient availability. However, biochar may also stimulate microbial activity, potentially accelerating native soil organic matter (OM) decomposition and altering soil OM molecular composition. Here we compare changes in soil microbial activity and native OM composition in a P-limited temperate hardwood forest in Ontario, Canada three years after the amendment of biochar and P fertilizer using a factorial design experiment. Phospholipid fatty acid analysis indicated that fungal activity was stimulated by biochar and biochar + P addition but not P amendment alone, while bacterial activity increased with all treatments. Concentrations of solvent-extractable acyclic and cyclic lipids, base-hydrolyzable cutin and suberin components, and lignin-

25 derived phenols increased with biochar and biochar + P amendment and to a lesser extent with P
26 fertilization. Biomarker ratios indicated soil OM compositional shifts toward greater proportions of
27 cyclic vs. acyclic aliphatic lipids and lignin-derived phenol monomers vs. dimers with biochar and
28 biochar + P amendment, but not with P fertilization. Solution-state nuclear magnetic resonance analysis
29 of base-extractable soil OM showed increased proportions of aliphatic lipids and lignin and fewer
30 carbohydrates with all treatments. The results suggest that biochar amendment may alleviate nutrient
31 deficiencies in P-limited forest ecosystems. However, biochar altered the soil microbial community
32 structure and shifted the native soil OM composition toward a greater proportion of recalcitrant OM,
33 which may alter soil OM turnover and nutrient cycling rates with long-term biochar amendment.

34

35 **Keywords**

36 Soil fertility, biomarkers, nuclear magnetic resonance, phospholipid fatty acids, suberin, cutin, lignin

37

38 **Introduction**

39 Nutrient limitation in temperate forest soils is a major impediment to plant growth and soil
40 fertility (Gress et al. 2007). Forest soil nutrient deficiencies may be addressed through the addition of N
41 and P fertilizers or lime (Sullivan et al. 2014; Moore et al. 2015), but the application of these
42 amendments may be problematic in the long-term. For example, fertilizers are costly and thus may have
43 important implications for the sustainability and economics of forest management (Cole 1995; Hedwall
44 et al. 2014). These amendments may also require repeated or frequent application (Brockley 2007;
45 Hedwall et al. 2010; Bergh et al. 2014), and nutrient runoff can lead to a reduction in water quality in
46 nearby watersheds (Fenn et al. 1998; Binkley et al. 1999). Furthermore, fertilizer and lime application
47 have been linked to increased fluxes of soil greenhouse gases such as CO₂ and N₂O (Sitaula et al. 1995;
48 West and McBride 2005). Soil amendment with biochar, the aromatic C-rich solid product resulting

49 from the pyrolysis of organic material (Lehmann and Joseph 2009), has been demonstrated to increase
50 soil water-holding and cation exchange capacities, buffer the pH of acidic soils, and sorb phytotoxic
51 compounds (Biederman and Harpole 2013). In addition, biochar can persist for centuries to millennia in
52 the environment, and the conversion of labile biomass C to biochar may increase soil C storage and
53 mitigate increasing atmospheric CO₂ concentrations that contribute to global climate change (Lehmann
54 2007). Studies examining biochar application to soil have primarily focused on agricultural ecosystems
55 due to the economic and societal benefits associated with improved crop yields (Jeffery et al. 2011). In
56 comparison, less research has focused on biochar addition to forests, especially in temperate ecosystems
57 (Gundale and DeLuca 2006; Santos et al. 2012; Singh et al. 2014; Noyce et al. 2015; Sackett et al.
58 2015). However, biochar application to forest soils may provide similar benefits as in agricultural
59 systems. For example, biochar may increase soluble ion concentrations in nutrient-deficient forest soils
60 (Thomas and Gale 2015; Zhu et al. 2015) through both the sorptive retention of cations and the addition
61 of feedstock-derived inorganic ash components (Lehmann et al. 2011).

62 Previous studies have reported stimulated soil microbial activity (increased soil CO₂ respiration)
63 following biochar amendment (Steinbeiss et al. 2009; Ameloot et al. 2013; Gomez et al. 2014), and
64 shifts in the microbial community composition of biochar-amended soils have been observed using
65 phospholipid fatty acid (PLFA) biomarkers (Prayogo et al. 2014; Watzinger et al. 2014; Mitchell et al.
66 2015). In contrast to fertilizer addition which may stimulate microbial activity by directly alleviating
67 nutrient deficiencies (Cleveland et al. 2002; Zhu et al. 2015), biochar likely enhances microbial activity
68 as a result of several favourable changes to soil properties. For example, biochar may improve soil
69 physical structure and aeration, increase soil nutrient concentrations and pH, as well as provide
70 macroporous environments that favour microbial colonization and offer microbes protection from
71 predators (Lehmann et al. 2011; McCormack et al. 2013; Gul et al. 2015). Since microbes are the
72 primary decomposers of organic matter (OM) in soil (Sheibani et al. 2013), biochar-induced changes in

73 microbial activity and community composition may alter soil C turnover. For example, both positive and
74 negative priming of soil C has been reported during biochar amendment, which may decrease or
75 increase native soil C stocks, respectively (Cross and Sohi 2011; Keith et al. 2011; Luo et al. 2011;
76 Zimmerman et al. 2011; Maestrini et al. 2014). However, it remains unclear how the overall
77 composition of native soil OM is altered by biochar addition to soil, including changes in the
78 proportions of specific types of OM components. For example, enhanced microbial activity may
79 decrease the concentration of labile, easily-degradable OM components such as sugars, peptides and
80 protein, resulting in an accumulation of more recalcitrant OM constituents such as long-chain aliphatic
81 lipids (von Lützow et al. 2006; Mitchell et al. 2016). Since soil OM plays a critical role in global
82 biogeochemical cycles (Lal 2008), it is important to assess whether biochar alters the native soil OM
83 composition to further evaluate the feasibility of applying biochar to nutrient-limited forest soils.

84 This study focuses on a three-year, field-scale amendment experiment conducted in a mixed
85 hardwood temperate forest located in central Ontario, Canada, in the Great Lakes-St. Lawrence region.
86 This area receives high levels of N deposition as a result of anthropogenic emissions in eastern North
87 America (Environment Canada 2005; Vet and Ro 2008). Previous studies have reported evidence of
88 essential plant nutrient deficiencies in soils of this region, primarily P, while K, Ca and Mg may be
89 limiting secondarily (Gradowski and Thomas 2006; Casson et al. 2012). P fertilizer and lime application
90 may be used to correct the altered soil nutrient ratios which result from elevated N inputs (Gradowski
91 and Thomas 2006; Peng and Thomas 2010; Moore and Ouimet 2014; Moore et al. 2015; Sackett et al.
92 2015). Biochar amendment may also provide long-term supplementation of these limiting soil nutrients
93 through the slow release and sorptive retention of ions (Mukherjee and Zimmerman 2013). Furthermore,
94 if biochar is prepared on-site from forest biomass, it may be a more cost-effective amendment than
95 fertilizer with the benefit of enhancing soil C sequestration (Thomas and Gale 2015). Previous studies at
96 this forest reported that biochar addition (5 t/ha) increased soil CO₂ respiration as well as the

97 concentration of several limiting soil nutrients, including PO_4^{3-} , K^+ , Ca^{2+} and Mg^{2+} , whereas P
98 fertilization (200 kg/ha) decreased the abundance of mycorrhizal fungi in the mineral soil as a result of
99 alleviated nutrient deficiencies after only one year (Noyce et al. 2015; Sackett et al. 2015). To further
100 investigate potential changes in the soil microbial community composition and soil OM chemistry with
101 P fertilization and different biochar application rates over a longer time scale, the present study used a
102 factorial design setup to compare soil responses to biochar (0, 5, 10 and 20 t/ha) and P (0 or 200 kg/ha)
103 amendments separately as well as to a combined biochar + P treatment three years post-amendment.
104 Shifts in the soil microbial community structure were examined using PLFAs (Frostegård and Bååth
105 1996), while changes in the native soil OM composition were assessed using soil OM biomarker and
106 solution-state nuclear magnetic resonance (NMR) spectroscopy techniques (Feng and Simpson 2011).
107 We hypothesize that biochar and P amendment in this forest soil will increase soil fertility by directly
108 providing available forms of P and other limiting plant nutrients, leading to increased bacterial and
109 fungal activity and enhanced mineralization (positive priming) of forest floor litter and native soil OM.
110 Our overall research objective is to examine biochar- vs. P-induced changes in microbial activity and
111 soil OM composition using the aforementioned molecular-level techniques to evaluate the potential
112 advantages and disadvantages of using biochar as a soil amendment in a nutrient-limited managed forest
113 ecosystem.

114

115 **Materials and methods**

116 Experimental design and sample collection

117 The biochar and P amendment study was conducted at the Haliburton Forest and Wild Life
118 Reserve Ltd. in central Ontario, Canada (45.29° N, 78.64° W). This temperate hardwood forest contains
119 primarily deciduous trees and is dominated by sugar maple (*Acer saccharum* Marshall). The mean
120 annual temperature and precipitation at the site are 1075 mm and 5 °C, respectively (Environment

Canada 2015; Sackett et al. 2015). The soil at the study site is classified as a Brunisol under the Canadian System of Soil Classification, an Inceptisol under the USDA Soil Taxonomy system and a Cambisol under the FAO World Reference Base soil classification system (Soil Classification Working Group 1998). The experiment was designed to test two factors: different biochar application rates (0, 5, 10 and 20 t/ha) and P amendment (unfertilized or fertilized with $\text{Ca}(\text{H}_2\text{PO}_4)_2$ at a rate of 200 kg/ha). The biochar application rates were selected based on previously published biochar amendment studies in temperate ecosystems (Steinbeiss et al. 2009; Smith et al. 2010; Case et al. 2012; Nelissen et al. 2012; Ameloot et al. 2013). The P application rate was based on previous research which assessed both microbial and plant responses to soil P fertilization at the Haliburton Forest (Gradowski and Thomas 2008; Noyce et al. 2015; Sackett et al. 2015). Using a factorial design, a total of 8 treatments were tested with five replicate plots per treatment, for a total of 40 experimental plots. The plots were 1×1 m in size and were delineated within a 60×20 m area of the forest in September 2011 using a randomized block design. Sugar maple sawdust (particle size range of 0.5-5 mm diameter) was pyrolyzed in several batches on-site using a small-scale, 80 L capacity rotating kiln heated by a flame to maximum treatment temperatures between 350-450 °C, with an average maximum temperature of 400 °C. The temperature inside the pyrolyzer was measured by a high-temperature thermocouple inserted into the center of the reaction chamber. During pyrolysis, the samples were held at the maximum temperature for approximately 40 min to ensure thorough pyrolysis and all resulting batches were combined and mixed prior to biochar application (Sackett et al. 2015). The biochar and P amendments were applied directly to the forest floor litter-fibric-humic (LFH) layer by hand prior to leaf fall and were not tilled into the soils to avoid damaging plant roots. Following application, the experimental plots were left undisturbed until the time of sampling and were covered by three consecutive years of autumn leaf fall. Soil samples were obtained in September 2014 by gently removing a small amount of the forest floor leaf litter at each plot by hand and then collecting duplicate soil cores (2 cm diameter), which were separated into

145 upper (0-5 cm) and lower (5-15 cm) mineral soil layers. All soil samples were stored at 4 °C during
146 transport to the laboratory, frozen within 8 h of collection and freeze-dried. The samples were 1 mm
147 sieved to remove rocks and visible plant material and homogenized using a mortar and pestle.
148 Preliminary elemental C and N analysis and solvent extraction showed similar elemental and biomarker
149 composition, respectively, in control soils across the five blocks (data not shown). As such, composite
150 soil samples were prepared by combining equal amounts of the ten replicate soil cores from the same
151 experimental treatment and sampling depth (4 biochar application rates $\times$ 2 P application rates $\times$ 2
152 depths, for a total of 16 composites). The composite samples were further homogenized using a mortar
153 and pestle prior to analysis.

154

155 Soil and biochar characterization

156 To measure pH, soil or biochar was mixed with deionized H₂O (1:10 w/v) on a reciprocal shaker
157 for 1 h (Hendershot et al. 2008), centrifuged, and the pH of the supernatant was measured using an
158 Accumet Basic pH electrode (Fisher Scientific). Total C and N content were determined by combusting
159 the soil and biochar samples at 950 °C using a Thermo Flash 2000 CN combustion analyzer and
160 measuring the evolved gases using a thermal conductivity detector. Organic carbon (OC) content was
161 determined by combusting the samples at 475 °C, while the inorganic C content was calculated as the
162 difference between the total C and OC content. Since inorganic C was not previously detected in
163 Haliburton Forest soils (Mitchell et al. 2015), the percentage of OC was equated to the total C content.
164 Extractable PO₄³⁻ was measured by extracting soils with 0.5 M sodium bicarbonate solution (1:20 w/v,
165 pH = 8.5) followed by colourimetric detection (Olsen et al. 1954). To examine potential nutrient losses
166 from the amended soils during the three year experiment, the expected increases in soil C and N content
167 and extractable PO₄³⁻ concentration at the 0-5 cm depth were calculated based on the chemical

168 properties and application rate of the biochar and P amendments, assuming homogeneous mixing of the
169 amendments into the upper mineral soil.

170

171 Soil OM biomarker extractions and solution-state ^1H NMR spectroscopy analysis

172 Several chemical extraction methods were used to isolate soil OM biomarkers, including a
173 modified Bligh-Dyer extraction protocol to isolate PLFAs (Bligh and Dyer 1959; Frostegård and Bååth
174 1996), solvent extraction to extract unbound lipids and sugars (Otto and Simpson 2005), base hydrolysis
175 to cleave ester-bound wax, cutin and suberin components (Otto and Simpson 2006b) and CuO oxidation
176 to isolate lignin-derived phenols (Hedges and Ertel 1982; Otto and Simpson 2006a). A detailed
177 description of the biomarker extraction and quantification is listed in the Supplementary Material. A
178 sub-sample of the biochar composite used in the amendment study was subjected to all four biomarker
179 extraction protocols in triplicate and did not contain detectable quantities of any OM biomarkers (data
180 not shown). As such, all detected biomarkers are derived from the native soil OM. Whole soil samples
181 were also subjected to HF demineralization (Gonçalves et al. 2003) followed by exhaustive extraction
182 with NaOH to isolate base-extractable soil OM (Kelleher and Simpson 2006), which was subsequently
183 analyzed using one- and two-dimensional (1-D and 2-D) solution-state ^1H NMR spectroscopy (see
184 Supplementary Material). Table 1 summarizes the major soil OM components and parameters which
185 were examined in the upper mineral soil using the biomarker and NMR analyses as well as the
186 corresponding results and their biogeochemical interpretation.

187

188 Statistical analysis

189 Principal component analysis (PCA) was performed on biomarker concentrations using SPSS
190 Statistics software (v. 20, IBM) to screen for soil microbial community and OM compositional shifts.
191 No data transformation was required since all biomarker data were normally distributed. PCA was

192 conducted on the four individual biomarker data sets separately as well as all OM biomarker data
193 combined. To assess the statistical significance of biomarker concentrations and ratios in amended soils
194 relative to the unamended control, and to examine potential interactions between the biochar and P
195 treatments, a two-way analysis of variance (ANOVA) with Tukey's honest significant difference post-
196 hoc test was applied to biomarker data sets using OriginPro software (v. 8.0, OriginLab). A p -value $\leq$
197 0.05 was considered statistically significant. The ANOVA F -statistic and p -values obtained for
198 biomarkers in the 0-5 cm and 5-15 cm depth soils are summarized in Tables S1 and S2, respectively.

199

200 **Results**

201 Elemental composition and chemical properties of soils

202 The biochar had a near-neutral pH = 6.73 and was rich in OC (86.7 %) with a small amount of
203 inorganic C also present (0.2%; Table S3). Sackett et al. (2015) reported that this biochar composite
204 contained extractable K^+ , Ca^{2+} , Mg^{2+} and PO_4^{3-} ions which could be leached and provide a nutrient
205 source in the soil. Table S4 lists the soil pH values as well as the measured and expected total C and N
206 content and extractable PO_4^{3-} concentrations of the soil samples. Soil pH ranged from 4.10-4.57 across
207 all samples and did not show a consistent trend with any amendment or between the two depths. This
208 agrees with two previous studies in which biochar amendment at this site did not alter soil pH (Noyce et
209 al. 2015; Sackett et al. 2015). At the 0-5 cm depth, the total C content was elevated in all samples
210 containing biochar relative to the unamended soil, which was anticipated since biochar is a C-rich
211 amendment. The total C content also increased in the P-fertilized soil. Total N was higher in all
212 amended 0-5 cm soil samples relative to the unamended soil. The increases in the total C and N content
213 of the amended 0-5 cm soils were higher than the values expected based on the amount of C and N
214 added in the form of biochar. This suggests that soil C and N content increased three years post-
215 amendment in the upper mineral soil, and indicates that any losses of biochar from the amended soil

plots were minimal (Major et al. 2010). At the 5-15 cm depth, all biochar, P and biochar + P amendments increased soil C and N to a similar extent, likely due to increased root growth. Extractable PO_4^{3-} concentrations in the P- and biochar + P-fertilized soils were higher than the unamended soil at both depths, but were only marginally higher than the unamended soil with biochar amendment. The measured extractable PO_4^{3-} concentrations in the biochar-amended soils were in close agreement with the expected values, whereas PO_4^{3-} concentrations were lower than expected in the P- and biochar + P-amended soils. This decrease in extractable PO_4^{3-} concentration after three years for all soils amended with P fertilizer is likely due to PO_4^{3-} uptake by soil microbes and plant biomass and/or leaching into lower soil horizons (Bhatti et al. 1998; Cui et al. 2011).

225

Principal component analysis (PCA)

PCA scores plots for the PLFA and total soil OM biomarker concentrations are shown in Fig. 1, while the PCA scores plots for the different biomarker classes are shown in Fig. S1. The PLFA and total soil OM biomarker PCA scores plots exhibited clear separation between the upper and lower soil horizons along PC1, which explained 91.5% and 69.0 % of the variance, respectively (Fig. 1). In addition, the upper soil horizon PCA scores showed more variation between the different treatments than the lower soil horizon, suggesting greater differences in the soil microbial community and soil OM composition at the 0-5 cm depth than at 5-15 cm. The same trend of greater variation in soil OM composition at the upper depth was also observed for the PCA scores for the individual biomarkers (Fig. S1). Since PCA revealed distinct differences in soil microbial community and OM composition between the treatments, biomarker concentrations and ratios were examined in greater detail to gain more specific insight into these compositional shifts.

238

Phospholipid fatty acids (PLFAs)

240 PLFA analysis revealed that biochar, P and biochar + P amendment stimulated soil microbial
 241 activity and altered the microbial community structure to different extents. A total of 30 PLFAs were
 242 identified in the soil samples which were assigned to the following sources (Table S5): Gram-positive
 243 bacteria (*i14:0*, *i15:0*, *a15:0*, *i16:0*, *a16:0*, *i17:0* and *a17:0*), Gram-negative bacteria (*16:1 ω 7c*, *cy17:0*,
 244 *18:1 ω 7c* and *cy19:0*), actinomycetes (*10Me16:0*, *10Me17:0* and *10Me18:0*), fungi (*18:2 ω 6,9c*) and non-
 245 specific PLFAs (Zelles 1999). At the 0-5 cm depth, concentrations of Gram-positive, Gram-negative and
 246 actinomycete PLFAs increased with nearly all treatments, in most cases significantly (Fig. 2a-c and
 247 Table S6). At the same depth, fungal PLFA concentrations increased significantly with biochar and
 248 biochar + P addition but not with P fertilization (Fig. 2d). At the 5-15 cm depth, the fungal PLFA
 249 concentration decreased significantly with P fertilization but increased significantly with biochar
 250 amendment (Table S7). The ANOVA results showed a significant interaction between biochar and P in
 251 the biochar + P-amended soils for all PLFA biomarkers at both depths, with the exception of the fungal
 252 PLFA in the lower soil (Tables S1-S2). Several ratios of PLFAs were calculated to examine microbial
 253 community shifts and substrate limitations (Feng and Simpson 2009). At the 0-5 cm depth, the Gram-
 254 negative/Gram-positive ratio decreased with all treatments (Fig. 2e). At both depths, the ratio of
 255 bacterial/fungal PLFAs decreased in the biochar and biochar + P-amended soils, in most cases
 256 significantly (Fig. 2f and Table S7). Furthermore, the bacterial substrate limitation ratios of
 257 *cy17:0/16:1 ω 7c* and *cy19:0/18:1 ω 7c* (Guckert et al. 1986; Kieft et al. 1994; Bossio and Scow 1998)
 258 decreased significantly with most amendments in the upper mineral soil (Table S6).

259

260 Solvent-extractable biomarkers

261 Solvent extraction showed increased concentrations of several plant- and microbial-derived
 262 biomarkers as well as shifts in the native OM composition of the amended soils. This extraction yielded
 263 several classes of compounds (Table S8), including sugars (monosaccharides and disaccharides), acyclic

264 aliphatic lipids (*n*-alkanes, *n*-alkanols and *n*-alkanoic acids) and cyclic aliphatic compounds (steroids
265 and triterpenoids). Statistically significant interactions between the biochar and P treatments were
266 observed for most solvent-extractable biomarkers in the biochar + P-amended samples at both depths
267 (Tables S1-S2). The sugars identified are found in plants and microbes and thus are not source-specific,
268 but can still provide insight into soil biogeochemical processes (Otto and Simpson 2005). At the 0-5 cm
269 depth, total sugar concentrations increased significantly in the biochar-amended soils and with biochar +
270 P amendment at the 10 t/ha application rate (Fig. 3a and Table S9). At the 5-15 cm depth, P fertilization
271 significantly decreased sugar concentrations whereas biochar + P addition significantly increased sugars
272 at the 20 t/ha application rate (Table S10). The solvent-extractable acyclic aliphatic lipids included a
273 homologous series of *n*-alkanes, *n*-alkanols and *n*-alkanoic acids which were classified as short-chain (<
274 C₂₀) or long-chain ($\geq$ C₂₀) compounds that are derived predominantly from microbial and plant sources,
275 respectively (Lichtfouse et al. 1995; Otto and Simpson 2005). Short-chain *n*-alkanoic acid
276 concentrations increased with biochar and biochar + P addition at the upper depth, in some cases
277 significantly (Fig. 3b). At the same depth, concentrations of long-chain *n*-alkanes, *n*-alkanols and *n*-
278 alkanoic acids increased significantly with most biochar, P and biochar + P amendments (Fig. 3c-e). At
279 the lower depth, long-chain *n*-alkane, *n*-alkanol and *n*-alkanoic acid concentrations increased
280 significantly for some treatments but did not show any definitive trends (Table S10). The solvent-
281 extractable cyclic aliphatic compounds included steroids and triterpenoids derived from microbial and
282 plant sources (Table S8). At the 0-5 cm depth, total steroid concentrations increased significantly with
283 all amendments (Fig. 3f). Triterpenoid concentrations also increased with all amendments at this depth,
284 in most cases significantly (Fig. 3g). At the 5-15 cm depth, steroid concentrations increased significantly
285 with biochar amendment but decreased significantly with P fertilization (Table S10). The concentration
286 of the fungal-derived steroid ergosterol increased with biochar and biochar + P amendment at the upper
287 depth, in most cases significantly (Fig. 3h). At the lower depth, ergosterol concentrations increased with

all amendments, significantly in soils with 20 t/ha biochar (Table S10). Ratios of solvent-extractable biomarker concentrations were calculated to further assess changes in soil OM composition and degradation status (Otto and Simpson 2005). The ratio of acyclic/cyclic compounds decreased significantly at the 0-5 cm depth with all biochar and biochar + P treatments (Fig. 3i). At the 5-15 cm depth, P fertilization significantly increased the acyclic/cyclic lipid ratio and significantly decreased the ratio of sterols/sterones (Table S10).

294

Base-hydrolyzable lipids

Base hydrolysis revealed increased concentrations of monomeric components of plant waxes and the biopolymers cutin and suberin in the amended soils. This treatment isolated a series of ester-bound compounds from the soil samples (Table S11), including monomeric components of waxes, cutin and suberin (Kolattukudy and Espelie 1989; Otto and Simpson 2006b). At the 0-5 cm depth, total concentrations of base-hydrolyzable benzyl and phenol compounds increased with all amendments, in some cases significantly (Table S12). Total concentrations of other base-hydrolyzable compounds including *n*-alkanols, *n*-alkanoic acids and α -hydroxyalkanoic acids increased with all biochar and P treatments at both depths, but significantly in only a few instances. Total concentrations of cutin biomarkers, namely the mid-chain-hydroxy C₁₅ and C₁₆ *n*-alkanoic acids, increased at both depths with all treatments, in several cases significantly (Fig. 4a and Table S13). Similarly, the total concentration of suberin components, including long-chain ω -hydroxyalkanoic acids and α,ω -*n*-alkanedioic acids, also increased at both depths with most treatments (Fig. 4b). Two-way ANOVA revealed a statistically significant interaction between biochar and P for most base-hydrolyzable biomarkers at the lower depth, but only in a few instances for the upper soil (Tables S1-S2). The ratio of suberin/cutin biomarkers (Otto and Simpson 2006b) decreased slightly with all treatments at the upper depth (Fig. 4c), signalling a shift toward a greater proportion of cutin components in the amended soils. Cutin and suberin degradation

312 ratios ($\omega\text{-C}_{16}/\Sigma\text{C}_{16}$, $\omega\text{-C}_{18}/\Sigma\text{C}_{18}$ and $\Sigma\text{mid}/\Sigma\text{SC}$) did not change at either depth (Tables S12-S13),
313 indicating no enhanced cutin or suberin degradation during the study.

314

315 Lignin-derived phenols

316 Alkaline CuO oxidation showed increased concentrations of lignin-derived phenol monomers
317 and dimers as well as a shift in the relative proportion of these components in the biochar and biochar +
318 P-amended soils. This treatment yielded several types of lignin-derived compounds (Hedges and Ertel
319 1982; Otto and Simpson 2006a) including phenol monomers (vanillyl, syringyl and cinnamyl classes)
320 and dimers ($5,5'$, $\alpha,1$ -monoketone, $\alpha,2$ -methyl and $\beta,1$ -diketone classes; Table S14). Several benzyl
321 compounds were also identified which may be derived from lignin as well as other sources such as
322 protein (Goñi et al. 2000; Otto and Simpson 2006a). At the 0-5 cm depth, total concentrations of benzyl
323 compounds, phenol monomers (denoted as ΣVSC) and phenol dimers increased significantly in the
324 biochar-amended soils at the 5 and 20 t/ha application rates, while biochar + P amendment significantly
325 increased ΣVSC at the 10 t/ha application rate (Fig. 4d,e and Table S15). At the 5-15 cm depth, total
326 concentrations of benzyl compounds, ΣVSC and dimers decreased significantly with P and biochar + P
327 amendment (Table S16). A statistically significant interaction was observed between the biochar and P
328 treatments for most lignin-derived compounds in the biochar + P-amended soils at the upper depth,
329 whereas this interaction was significant in only a few cases at the lower depth (Tables S1-S2). Ratios of
330 lignin-derived phenol compounds were calculated to assess the lignin degradation status and the relative
331 proportion of monomeric and dimeric lignin components in the soils (Ertel and Hedges 1984; Hedges et
332 al. 1988; Goñi and Hedges 1992; Otto and Simpson 2006a). At the upper depth, acid/aldehyde ratios did
333 not change significantly with any treatment (Table S15). At the lower depth, the dehydrodivanillic
334 acid/dehydrodivanillin ratio increased significantly in the 10 and 20 t/ha biochar + P-amended soils
335 (Table S16). The ratio of lignin-derived phenol monomers/dimers increased with P fertilization and to a

336 greater extent with biochar and biochar + P amendment at the 0-5 cm depth, in several cases
337 significantly (Fig. 4f). Significant increases in this ratio were also seen with most treatments at the 5-15
338 cm depth (Table S16).

339

340 Solution-state NMR spectroscopy

341 Solution-state ^1H NMR analysis of soil base extracts revealed decreased proportions of labile *O*-
342 alkyl OM and increased proportions of more recalcitrant alkyl C-rich OM in the amended soils.
343 Representative 1-D and 2-D solution-state NMR spectra of the extracts with integrated areas labelled are
344 shown in Fig. 5 and the integration results are summarized in Tables S17-S19. At the 0-5 cm depth, 1-D
345 ^1H NMR analysis showed that the relative proportion of aliphatic components such as lipids, waxes,
346 cutin and suberin increased at the 20 t/ha biochar application rate both with and without P fertilization
347 (Table S17). The proportion of *N*- and *O*-substituted aliphatic components such as lipids, amino acids,
348 peptidoglycan and chitin increased marginally with P fertilization, but decreased with biochar and
349 biochar + P amendment. A slight decrease in *O*-alkyl constituents including carbohydrates, peptides and
350 amino acids was observed in the soils containing 20 t/ha biochar, while the proportions of aromatic and
351 phenolic components such as lignin and aromatic amino acids increased in the same samples. At the 5-
352 15 cm depth, P fertilization increased the amount of *N*- and *O*-substituted aliphatic components and
353 decreased the amount of *O*-alkyl compounds. 1-D DE ^1H NMR was used to analyze the composition of
354 macromolecules and rigid OM components as this technique attenuates the signals from small molecules
355 that diffuse quickly in solution (Simpson et al. 2007). Increased proportions of macromolecular aliphatic
356 constituents such as waxes, cutin and suberin were observed with all treatments at both depths, with the
357 greatest increases at the 20 t/ha biochar application rate (Table S18). A pronounced decrease in large *O*-
358 alkyl structures such as carbohydrates, peptides and protein was seen at the 0-5 cm depth with biochar
359 and biochar + P amendment. At the same depth, increased proportions of aromatic and phenolic

360 components were observed at the 10 and 20 t/ha application rates, while a similar increase was noted at
361 the 5-15 cm depth but only with 20 t/ha biochar addition. 2-D ^1H - ^{13}C HSQC NMR spectroscopy was
362 used to confirm 1-D spectral assignments as this technique provides enhanced resolution of OM
363 components due to the greater spectral dispersion of the ^{13}C nucleus (Simpson et al. 2011). Changes in
364 the relative proportions of specific OM functionalities were assessed by calculating normalized area
365 ratios for defined regions of the HSQC spectra (Table S19 and Fig. 5c). At the 0-5 cm depth, the relative
366 proportion of aliphatic constituents rich in CH_2 and CH_3 groups increased with all treatments compared
367 to the unamended soil. Increased proportions of peptide-derived components such as α -protons and the
368 amino acids phenylalanine and tyrosine as well as higher proportions of lignin methoxy groups were
369 also observed at this depth with most treatments, in particular at the two higher biochar application rates.
370 Carbohydrate signals including CH , CH_2 and anomeric groups decreased with most treatments at both
371 depths, especially at the 10 and 20 t/ha biochar application rates both with and without P fertilization.

372

373 **Discussion**

374 Biochar and P amendment stimulated soil microbial activity and altered the soil microbial community
375 composition

376 The results suggest that biochar and P fertilizer addition to a P-limited temperate forest soil
377 resulted in some similar but also contrasting changes in soil microbial activity three years post-
378 amendment. The increased concentrations of PLFAs specific to Gram-positive and Gram-negative
379 bacteria and actinomycetes that were observed in the upper mineral soil (0-5 cm) signify that bacterial
380 activity was stimulated by most biochar and P amendments. However, the lack of significant differences
381 between the treatments indicates that bacterial activity was not preferentially stimulated by one
382 amendment over the others. The higher concentrations of microbial-derived short-chain *n*-alkanoic acids
383 observed in the soil solvent extracts (Harwood and Russell 1984; Lichtfouse et al. 1995) also suggest

384 increased microbial-derived inputs to the amended soils. Previous studies have also reported stimulated
385 bacterial activity following biochar amendment in soil using PLFA analysis (Steinbeiss et al. 2009;
386 Santos et al. 2012; Gomez et al. 2014; Prayogo et al. 2014; Watzinger et al. 2014; Ameloot et al. 2015;
387 Mitchell et al. 2015). This increase in bacterial activity over time may have arisen from biochar-induced
388 improvements to soil properties such as nutrient availability and macroporous habitats for microbes
389 (Mukherjee and Zimmerman 2013; Brewer et al. 2014; Gul et al. 2015). Since biochar, P and biochar +
390 P stimulated bacterial activity to a similar extent, this enhanced activity was likely the result of increased
391 soil nutrient availability rather than the presence of the biochar particles as a physical substrate. This is
392 supported by decreases in the PLFA stress ratios, which indicated that bacteria experienced less
393 substrate constraints in the amended soils (Guckert et al. 1986; Kieft et al. 1994; Bossio and Scow
394 1998). Lower ratios of Gram-negative/Gram-positive bacterial PLFAs at the 0-5 cm depth with all
395 biochar and P amendments suggest that the bacterial community in the amended soils shifted toward a
396 greater proportion of Gram-positive bacteria. We previously reported the preferential stimulation of
397 Gram-positive bacteria over time during a laboratory study in which sugar maple wood-derived biochar
398 was amended in soil collected from the same forest (Mitchell et al. 2015). Other researchers have also
399 observed a bacterial community shift toward Gram-positive bacteria during a short-term amendment
400 study using a different soil type and biochar (Ameloot et al. 2013). Gul et al. (2015) proposed that
401 Gram-positive bacteria preferentially utilize biochar-derived C as an energy source compared to Gram-
402 negative bacteria since Gram-positive bacteria have been found to selectively degrade more recalcitrant
403 forms of soil OM. For example, one study reported that the activity of Gram-positive bacteria increased
404 with soil depth as concentrations of recalcitrant OM components increased (Fierer et al. 2003). Two
405 previous biochar amendment studies also reported that Gram-positive bacteria incorporated biochar-
406 derived C into their cell membrane lipids (Santos et al. 2012; Farrell et al. 2013). Given that bacteria in
407 the surface mineral soil would be in close proximity to the amended biochar particles, a shift toward

Gram-positive bacteria could be expected at this depth. In addition, a shift toward Gram-positive bacteria was not observed in the lower soil layer which did not contain visible biochar particles. Stimulation of Gram-positive bacteria by P fertilization of forest soils has also been reported previously, albeit primarily in P-limited tropical ecosystems (Krashevskaya et al. 2010; Fanin et al. 2015). Collectively, our results suggest that biochar, P and biochar + P addition at this site favoured Gram-positive bacteria and caused localized shifts in the soil bacterial community structure.

The fungal PLFA (18:2 ω 6,9c) and ergosterol data indicated that fungal activity in the upper mineral soil was stimulated by biochar and biochar + P addition but not by P fertilization alone, in contrast with bacterial activity which increased with all amendments at this depth. This suggests that fungal activity may not be dependent on soil P concentrations to the same extent as bacteria, and that fungi responded favourably to biochar addition. Previous studies have reported that fungi can colonize biochar particles and macropores via their hyphal networks, potentially allowing access to a concentrated source of biochar-derived nutrients and residual pyrolysis compounds as well as a physical substrate to support growth (Ascough et al. 2010; Spokas et al. 2011; Hammer et al. 2014; Jaafar et al. 2014). The fungal PLFA and ergosterol data also showed that fungal activity was stimulated in the lower mineral soil when biochar was applied to the forest floor. Fungal hyphae may have accessed biochar particles at the soil surface which would be inaccessible to bacteria in the deeper soil. Conversely, fungal activity decreased at the lower depth with P fertilization. This was likely due to a reduction in mycorrhizal interactions with plant roots due to increased P availability (van der Heijden et al. 2008), and is consistent with a previous P fertilization experiment that was conducted at this forest site (Noyce et al. 2015). The decreasing bacterial/fungal PLFA ratio at both soil depths with biochar and biochar + P amendment indicates that fungal activity was stimulated to a greater extent than bacterial activity, and that the microbial community shifted toward a greater proportion of fungi vs. bacteria with these treatments. This suggests that the modified soil conditions with biochar were more favourable for fungi

432 than bacteria, and agrees with two previous studies which also observed preferential stimulation of soil
433 fungi in response to long-term biochar addition (Steinbeiss et al. 2009; Farrell et al. 2013). The shifts in
434 microbial community composition observed with the biochar and biochar + P amendments may have
435 ecological implications as microbes play a critical role in soil processes such as OM decomposition,
436 nutrient cycling and greenhouse gas production, among others (Thies and Rillig 2009). As such,
437 potential shifts in soil microbial communities should be carefully assessed prior to large-scale biochar
438 amendment of forest soils.

439

440 Biochar and P amendment altered the native soil OM molecular composition

441 Biomarker analysis revealed that several types of plant- and microbial-derived OM components
442 accumulated in the 0-5 cm amended soils during the three-year period. This included solvent-extractable
443 short and long-chain aliphatic lipids, sugars, steroids and triterpenoids, base-hydrolyzable cutin and
444 suberin components, as well as lignin-derived phenol monomers and dimers. Higher proportions of alkyl
445 polymethylene and lignin methoxy group resonances in the solution-state NMR spectra of the amended
446 soil base extracts further confirmed the increased inputs of plant-derived OM. Since the PLFA results
447 indicated that bacterial activity in the upper mineral soil was stimulated by P fertilization, we propose
448 that enhanced microbial decomposition of forest floor litter resulted in increased inputs of extractable
449 plant- and microbial-derived biomarkers in the P-fertilized soil. An increase in the total C and N content
450 of the P-amended soil relative to the unamended soil supports this potential accumulation of soil OM
451 components. For soils amended with biochar and biochar + P, the higher extractable biomarker
452 concentrations could be due to increased plant and microbial inputs as well as the sorptive retention of
453 biomarker compounds by biochar particles, which have a strong sorption capacity for OM (Uchimiya et
454 al. 2010; Smebye et al. 2016). Sorption to biochar may lower the bioavailability and thus the
455 susceptibility of these OM components to microbial degradation, potentially increasing their soil

456 residence time (Chenu and Plante 2006; Mikutta et al. 2007) and resulting in higher concentrations of
457 extractable biomarkers. Collectively, higher OM inputs and the sorptive preservation of OM on biochar
458 particles may explain why the total C and N content of the biochar and biochar + P-amended soils were
459 higher than predicted based on the amount of C and N added in the form of biochar. While the biochar
460 and biochar + P treatments increased the concentration of most extractable soil OM biomarkers, P
461 fertilization led to an accumulation of primarily slower-degrading OM components such as steroids,
462 triterpenoids, cutin and suberin. A previous P fertilization experiment at the same forest demonstrated
463 that concentrations of soil PO_4^{3-} , K^+ , Ca^{2+} and Mg^{2+} increased within 2-6 weeks of P fertilization at the
464 same application rate (Sackett et al. 2015). This increased nutrient availability may have stimulated
465 microbial activity and induced soil priming earlier in the study, leading to increased soil OM
466 degradation and preferentially mineralizing more labile OM components such as sugars. This may
467 explain why the concentrations of the more recalcitrant compounds increased after three years in the P-
468 fertilized plots, while concentrations of more labile biomarkers were unchanged.

469 Analysis of soil OM biomarker ratios and solution-state NMR data revealed several potential
470 shifts in the OM composition of the amended soils at the 0-5 cm depth. Although both acyclic and cyclic
471 aliphatic lipids accumulated, a decreased acyclic/cyclic lipid ratio with all biochar and biochar + P
472 treatments indicated an OM compositional shift toward a greater proportion of steroid and triterpenoid
473 compounds in these soils. Stimulated microbial activity may have resulted in the preferential
474 degradation of acyclic aliphatic lipids such as *n*-alkanes, *n*-alkanols and *n*-alkanoic acids compared to
475 cyclic lipids, which may be more inherently resistant to degradation due to their molecular structure
476 (von Lützow et al. 2006). For example, plant sterols are commonly found in soils in an unaltered state
477 and in greater abundance than their sterone oxidation products (Otto and Simpson 2005). The
478 preferential sorption of cyclic compounds to biochar particles may also account for their relative
479 preservation compared to the acyclic aliphatic lipids, especially since this OM compositional shift was

not observed with P fertilization. CuO oxidation revealed a shift toward a greater proportion of extractable lignin-derived phenol monomers vs. dimers in the biochar- and biochar + P-amended soils at the upper depth. Lignin is bound to hemicellulose and cellulose in plant cell walls, forming the ligno-cellulose complex (Sjöström 1993), and the degradation of ligno-cellulose in soil is believed to be initiated primarily by fungi as well as some species of bacteria (Antai and Crawford 1981; Hedges et al. 1988; Orth et al. 1993). These microbes are capable of cleaving lignin from the polysaccharide, which may in turn increase substrate accessibility to other lignin-degrading fungi and bacteria (Tuomela et al. 2000). Since the PLFA and ergosterol data suggested that fungal activity was stimulated by the biochar and biochar + P treatments, increases in the lignin monomer/dimer ratio may reflect the preferential degradation of dimeric lignin subunits by microbes (Goñi et al. 1993), or changes in the relative extractability of lignin sidechains due to structural alteration of the lignin macromolecule. Solution-state NMR analysis of soil base extracts showed an OM compositional shift toward a lower proportion of carbohydrate-derived components and a higher proportion of alkyl and lignin constituents with all amendments. Decreased proportions of carbohydrates may indicate enhanced microbial degradation of polysaccharides such as cellulose, which is supported by the higher concentrations of solvent-extractable sugar monomers that were observed in the amended soils. The concomitant increases in alkyl and lignin-derived compounds are consistent with the accumulation of aliphatic lipids and lignin phenols that were detected using biomarker analysis, and with a previous laboratory biochar incubation study conducted using soil from this forest (Mitchell et al. 2016).

Soil OM composition in the biochar-amended soils did not vary as greatly from the control at the 5-15 cm depth as in the surface mineral soil, likely because the biochar was not thoroughly mixed into the lower depth upon application. However, lower concentrations of sugars, long-chain *n*-alkanes and *n*-alkanoic acids, steroids and triterpenoids as well as lignin-derived phenols were found in the lower mineral soil with P fertilization. The sterol/sterone ratio also decreased in this soil, which indicates that

sterols were at a more advanced stage of oxidation. These findings suggest increased soil OM degradation in the lower soil horizon three years post-amendment, but the PLFA data indicated that microbial activity in the P-amended soil was similar to or lower than the unamended soil at this depth. The decrease in OM components may be explained by soil priming shortly after the P fertilizer was added. P leaching downward from the surface soil may have stimulated microbial activity (Cleveland et al. 2002; Zhu et al. 2015) and led to accelerated OM decomposition in the lower mineral soil, resulting in a decrease in the concentration of specific OM components. Microbial activity in the 5-15 cm soil may have then decreased to the observed levels once amended P concentrations were depleted by leaching and/or incorporation into soil organisms (Bhatti et al. 1998; Cui et al. 2011).

513

Biochar and P fertilizer as prospective soil amendments in P-limited managed forests

This study demonstrated that biochar, P and biochar + P amendment in a P-limited temperate forest for three years increased soil nutrient availability, altering the soil microbial community and native soil OM composition as hypothesized. In several instances, biochar, P and biochar + P addition led to similar changes in these soil properties. For example, all three types of amendments stimulated soil bacterial activity and increased the concentration of extractable plant-derived soil OM biomarkers, likely due to the enhanced breakdown of forest floor litter by microbes. However, several important differences were also observed between the biochar and P amendments, which may have implications for soil C sequestration and nutrient supplementation. First, P fertilization appears to have induced soil priming since labile OM components such as solvent-extractable sugars did not accumulate in this soil, whereas labile OM constituents accumulated with biochar amendment. Although P fertilization may increase the availability of limiting soil nutrients, fertilizer application may be disadvantageous in the long-term from a C sequestration perspective as priming may enhance soil OM decomposition and soil CO₂ efflux (Kuzyakov et al. 2000; Keith et al. 2011; Luo et al. 2011; Zimmerman et al. 2011). Secondly,

528 soil OM compositional shifts toward a higher concentration of cyclic vs. acyclic lipids and lignin-
529 derived phenol monomers vs. dimers were observed with biochar amendment but not with P
530 fertilization. These compositional shifts suggest that biochar amendment may result in the preferential
531 accumulation of specific OM components. This may be due to the sorption of OM onto biochar particles
532 (Smebye et al. 2016), which could increase soil C storage but may also alter soil C biogeochemical
533 cycling. Thirdly, biochar amendment stimulated soil fungal activity and shifted the microbial
534 community toward a greater proportion of fungi over bacteria, which was not observed with P
535 fertilization alone. Since fungi are important decomposers in soil, long-term microbial community shifts
536 toward fungi with biochar addition may increase soil nutrient availability (McTiernan et al. 1997;
537 Kalbitz et al. 2000; Lehmann et al. 2011). However, increased fungal activity could further shift the
538 native soil OM composition, and it is unclear whether biochar amendment stimulated the activity of
539 fungi which are plant pathogens (Elad et al. 2010; Graber et al. 2014). In most cases, the biochar and
540 biochar + P amendments enhanced microbial activity and altered the native soil OM composition to a
541 similar extent. Although the soils at this site are reported to be P-deficient (Gradowski and Thomas
542 2006; Casson et al. 2012; Sackett et al. 2015), it appears that the amount of P and other ionic nutrients
543 supplied by biochar amendment was sufficient to alleviate soil nutrient deficiencies and enhance
544 microbial activity. Overall, it may be advantageous to use biochar as an amendment in P-limited
545 managed forests as biochar provides a sustained source and sorbent of nutrients without increasing soil
546 priming. However, long-term monitoring of nutrient availability in the biochar-amended soils is needed
547 to further assess the nutrient supplementation capability of biochar in this forest ecosystem over longer
548 time scales. Furthermore, potential biochar-induced changes in soil OM biogeochemistry should be
549 studied in other managed forests to better understand the ramifications of biochar addition prior to the
550 amendment of unmanaged forests.

551

552 Acknowledgements

553 This work was funded by a Strategic Projects Grant from the Natural Sciences and Engineering
554 Research Council (NSERC) of Canada. P.J.M. thanks NSERC for a Postgraduate Scholarship, H.
555 Masoom and K. Gaynor for assistance with soil sampling, G. Noyce and N. Wagner for advice on
556 statistics, and the Haliburton Forest and Wild Life Reserve Ltd. for study site access.

558 Electronic supplementary material

559 The online version of this article contains supplementary material, which is available to
560 authorized users.

562 References

- 563 Ameloot N, De Neve S, Jegajeevagan K, Yildiz G, Buchan D, Funkuin YN, Prins W, Bouckaert L,
564 Sleutel S (2013) Short-term CO₂ and N₂O emissions and microbial properties of biochar amended
565 sandy loam soils. *Soil Biol Biochem* 57:401-410
- 566 Ameloot N, Sleutel S, Das KC, Kanagaratnam J, De Neve S (2015) Biochar amendment to soils with
567 contrasting organic matter level: Effects on N mineralization and biological soil properties. *GCB*
568 *Bioenergy* 7:135-144
- 569 Antai SP, Crawford DL (1981) Degradation of softwood, hardwood, and grass lignocelluloses by two
570 *Streptomyces* strains. *Appl Environ Microbiol* 42:378-380
- 571 Ascough PL, Sturrock CJ, Bird MI (2010) Investigation of growth responses in saprophytic fungi to
572 charred biomass. *Isotopes Environ Health Stud* 46:64-77
- 573 Bergh J, Nilsson U, Allen H, Johansson U, Fahlvik N (2014) Long-term responses of Scots pine and
574 Norway spruce stands in Sweden to repeated fertilization and thinning. *For Ecol Manage* 320:118-
575 128
- 576 Bhatti JS, Comerford NB, Johnston CT (1998) Influence of oxalate and soil organic matter on sorption
577 and desorption of phosphate onto a spodic horizon. *Soil Sci Soc Am J* 62:1089-1095
- 578 Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: A
579 meta-analysis. *GCB Bioenergy* 5:202-214
- 580 Binkley D, Burnham H, Lee Allen H (1999) Water quality impacts of forest fertilization with nitrogen
581 and phosphorus. *For Ecol Manage* 121:191-213
- 582 Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem*
583 *Physiol* 37:911-917
- 584 Bossio DA, Scow KM (1998) Impacts of carbon and flooding on soil microbial communities:
585 Phospholipid fatty acid profiles and substrate utilization patterns. *Microb Ecol* 35:265-278
- 586 Brewer CE, Chuang VJ, Masiello CA, Gonnermann H, Gao X, Dugan B, Driver LE, Panzacchi P,
587 Zygourakis K, Davies CA (2014) New approaches to measuring biochar density and porosity.
588 *Biomass Bioenergy* 66:176-185

589 Brockley RP (2007) Effects of 12 years of repeated fertilization on the foliar nutrition and growth of
590 young lodgepole pine in the central interior of British Columbia. *Can J For Res* 37:2115-2129

591 Case SDC, McNamara NP, Reay DS, Whitaker J (2012) The effect of biochar addition on N₂O and CO₂
592 emissions from a sandy loam soil - The role of soil aeration. *Soil Biol Biochem* 51:125-134

593 Casson NJ, Eimers MC, Watmough SA (2012) An assessment of the nutrient status of sugar maple in
594 Ontario: Indications of phosphorus limitation. *Environ Monit Assess* 184:5917-5927

595 Chenu C, Plante AT (2006) Clay-sized organo-mineral complexes in a cultivation chronosequence:
596 Revisiting the concept of the 'primary organo-mineral complex'. *Eur J Soil Sci* 57:596-607

597 Cleveland CC, Townsend AR, Schmidt SK (2002) Phosphorus limitation of microbial processes in
598 moist tropical forests: Evidence from short-term laboratory incubations and field studies.
599 *Ecosystems* 5:680-691

600 Cole DW (1995) Soil nutrient supply in natural and managed forests. *Plant Soil* 168-169:43-53

601 Cross A, Sohi SP (2011) The priming potential of biochar products in relation to labile carbon contents
602 and soil organic matter status. *Soil Biol Biochem* 43:2127-2134

603 Cui H, Wang MK, Fu M, Ci E (2011) Enhancing phosphorus availability in phosphorus-fertilized zones
604 by reducing phosphate adsorbed on ferrihydrite using rice straw-derived biochar. *J Soils*
605 *Sediments* 11:1135-1141

606 Elad Y, David DR, Harel YM, Borenshtein M, Kalifa HB, Silber A, Graber ER (2010) Induction of
607 systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. *Phytopathology*
608 100:913-921

609 Environment Canada (2005) Canadian Acid Deposition Science Assessment 2004. Environment
610 Canada. Retrieved from: <http://publications.gc.ca/site/eng/9.688243/publication.html>

611 Environment Canada (2015) Climate normals and averages, 1981-2010. Environment Canada. Retrieved
612 from: http://climate.weather.gc.ca/climate_normals/index_e.html

613 Ertel JR, Hedges JI (1984) The lignin component of humic substances: Distribution among soil and
614 sedimentary humic, fulvic, and base-insoluble fractions. *Geochim Cosmochim Acta* 48:2065-2074

615 Fanin N, Hättenschwiler S, Schimann H, Fromin N (2015) Interactive effects of C, N and P fertilization
616 on soil microbial community structure and function in an Amazonian rain forest. *Funct Ecol*
617 29:140-150

618 Farrell M, Kuhn TK, Macdonald LM, Maddern TM, Murphy DV, Hall PA, Singh BP, Baumann K,
619 Krull ES, Baldock JA (2013) Microbial utilisation of biochar-derived carbon. *Sci Total Environ*
620 465:288-297

621 Feng X, Simpson MJ (2009) Temperature and substrate controls on microbial phospholipid fatty acid
622 composition during incubation of grassland soils contrasting in organic matter quality. *Soil Biol*
623 *Biochem* 41:804-812

624 Feng X, Simpson MJ (2011) Molecular-level methods for monitoring soil organic matter responses to
625 global climate change. *J Environ Monit* 13:1246-1254

626 Fenn ME, Poth MA, Aber JD, Baron JS, Bormann BT, Johnson DW, Lemly AD, McNulty SG, Ryan
627 DF, Stottlemeyer R (1998) Nitrogen excess in North American ecosystems: Predisposing factors,
628 ecosystem responses, and management strategies. *Ecol Appl* 8:706-733

629 Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two
630 soil depth profiles. *Soil Biol Biochem* 35:167-176

631 Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and
632 fungal biomass in soil. *Biol Fertil Soils* 22:59-65

633 Gomez JD, Deneff K, Stewart CE, Zheng J, Cotrufo MF (2014) Biochar addition rate influences soil
634 microbial abundance and activity in temperate soils. *Eur J Soil Sci* 65:28-39

635 Gonçalves CN, Dalmolin RSD, Dick DP, Knicker H, Klamt E, Kögel-Knabner I (2003) The effect of
636 10% HF treatment on the resolution of CPMAS ¹³C NMR spectra and on the quality of organic
637 matter in Ferralsols. *Geoderma* 116:373-392

638 Goñi MA, Hedges JI (1992) Lignin dimers: Structures, distribution, and potential geochemical
639 applications. *Geochim Cosmochim Acta* 56:4025-4043

640 Goñi MA, Nelson B, Blanchette RA, Hedges JI (1993) Fungal degradation of wood lignins:
641 Geochemical perspectives from CuO-derived phenolic dimers and monomers. *Geochim*
642 *Cosmochim Acta* 57:3985-4002

643 Goñi MA, Yunker MB, MacDonald RW, Eglinton TI (2000) Distribution and sources of organic
644 biomarkers in arctic sediments from the Mackenzie River and Beaufort Shelf. *Mar Chem* 71:23-51

645 Graber ER, Frenkel O, Jaiswal AK, Elad Y (2014) How may biochar influence severity of diseases
646 caused by soilborne pathogens? *Carbon Manage* 5:169-183

647 Gradowski T, Thomas SC (2006) Phosphorus limitation of sugar maple growth in central Ontario. *For*
648 *Ecol Manage* 226:104-109

649 Gradowski T, Thomas SC (2008) Responses of *Acer saccharum* canopy trees and saplings to P, K and
650 lime additions under high N deposition. *Tree Physiol* 28:173-185

651 Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting
652 differing nitrogen availabilities: What lies beyond nitrogen saturation? *Ecology* 88:119-130

653 Guckert JB, Hood MA, White DC (1986) Phospholipid ester-linked fatty acid profile changes during
654 nutrient deprivation of *Vibrio cholerae*: Increases in the trans/cis ratio and proportions of
655 cyclopropyl fatty acids. *Appl Environ Microbiol* 52:794-801

656 Gul S, Whalen JK, Thomas BW, Sachdeva V, Deng H (2015) Physico-chemical properties and
657 microbial responses in biochar-amended soils: Mechanisms and future directions. *Agric Ecosyst*
658 *Environ* 206:46-59

659 Gundale MJ, DeLuca TH (2006) Temperature and source material influence ecological attributes of
660 ponderosa pine and Douglas-fir charcoal. *For Ecol Manage* 231:86-93

661 Hammer EC, Balogh-Brunstad Z, Jakobsen I, Olsson PA, Stipp SLS, Rillig MC (2014) A mycorrhizal
662 fungus grows on biochar and captures phosphorus from its surfaces. *Soil Biol Biochem* 77:252-
663 260

664 Harwood JL, Russell NJ (1984) *Lipids in Plants and Microbes*. George Allen and Unwin, London, UK

665 Hedges JI, Ertel JR (1982) Characterization of lignin by gas capillary chromatography of cupric oxide
666 oxidation products. *Anal Chem* 54:174-178

667 Hedges JI, Blanchette RA, Weliky K, Devol AH (1988) Effects of fungal degradation on the CuO
668 oxidation products of lignin: A controlled laboratory study. *Geochim Cosmochim Acta* 52:2717-
669 2726

670 Hedwall PO, Nordin A, Brunet J, Bergh J (2010) Compositional changes of forest-floor vegetation in
671 young stands of Norway spruce as an effect of repeated fertilisation. *For Ecol Manage* 259:2418-
672 2425

673 Hedwall PO, Gong P, Ingerslev M, Bergh J (2014) Fertilization in northern forests - biological,
674 economic and environmental constraints and possibilities. *Scand J For Res* 29:301-311

675 Hendershot WH, Lalande H, Duquette M (2008) Soil reaction and exchangeable acidity. In: Carter MR,
676 Gregorich EG (eds) *Soil Sampling and Methods of Analysis*, 2nd edn. CRC Press, Boca Raton,
677 FL, USA, pp 173-178

678 Jaafar NM, Clode PL, Abbott LK (2014) Microscopy observations of habitable space in biochar for
679 colonization by fungal hyphae from soil. *J Integr Agric* 13:483-490

680 Jeffery S, Verheijen FGA, van der Velde M, Bastos AC (2011) A quantitative review of the effects of
681 biochar application to soils on crop productivity using meta-analysis. *Agric Ecosyst Environ*
682 144:175-187

683 Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved
 684 organic matter in soils: A review. *Soil Sci* 165:277-304
 685 Keith A, Singh B, Singh BP (2011) Interactive priming of biochar and labile organic matter
 686 mineralization in a smectite-rich soil. *Environ Sci Technol* 45:9611-9618
 687 Kelleher BP, Simpson AJ (2006) Humic substances in soils: Are they really chemically distinct?
 688 *Environ Sci Technol* 40:4605-4611
 689 Kieft TL, Ringelberg DB, White DC (1994) Changes in ester-linked phospholipid fatty acid profiles of
 690 subsurface bacteria during starvation and desiccation in a porous medium. *Appl Environ Microbiol*
 691 60:3292-3299
 692 Kolattukudy PE, Espelie KE (1989) Chemistry, biochemistry and function of suberin and associated
 693 waxes. In: Rowe JW (ed) *Natural Products of Woody Plants*. Springer, Berlin, Germany, pp 304-
 694 367
 695 Krashevskaya V, Maraun M, Russ L, Scheu S (2010) Carbon and nutrient limitation of soil
 696 microorganisms and microbial grazers in a tropical montane rain forest. *Oikos* 119:1020-1028
 697 Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects.
 698 *Soil Biol Biochem* 32:1485-1498
 699 Lal R (2008) Sequestration of atmospheric CO₂ in global carbon pools. *Energy Environ Sci* 1:86-100
 700 Lehmann J, Joseph S (2009) Biochar for environmental management: An introduction. In: Lehmann J,
 701 Joseph S (eds) *Biochar for Environmental Management: Science and Technology*. Earthscan,
 702 London, UK, pp 1-12
 703 Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil
 704 biota - A review. *Soil Biol Biochem* 43:1812-1836
 705 Lehmann J (2007) A handful of carbon. *Nature* 447:143-144
 706 Lichtfouse É, Berthier G, Houot S, Barriuso E, Bergheaud V, Vallaëys T (1995) Stable carbon isotope
 707 evidence for the microbial origin of C₁₄-C₁₈ *n*-alkanoic acids in soils. *Org Geochem* 23:849-852
 708 Luo Y, Durenkamp M, De Nobili M, Lin Q, Brookes PC (2011) Short term soil priming effects and the
 709 mineralisation of biochar following its incorporation to soils of different pH. *Soil Biol Biochem*
 710 43:2304-2314
 711 Maestrini B, Herrmann AM, Nannipieri P, Schmidt MWI, Abiven S (2014) Ryegrass-derived pyrogenic
 712 organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil. *Soil*
 713 *Biol Biochem* 69:291-301
 714 Major J, Lehmann J, Rondon M, Goodale C (2010) Fate of soil-applied black carbon: Downward
 715 migration, leaching and soil respiration. *Global Change Biol* 16:1366-1379
 716 McCormack SA, Ostle N, Bardgett RD, Hopkins DW, Vanbergen AJ (2013) Biochar in bioenergy
 717 cropping systems: Impacts on soil faunal communities and linked ecosystem processes. *GCB*
 718 *Bioenergy* 5:81-95
 719 McTiernan KB, Ineson P, Coward PA (1997) Respiration and nutrient release from tree leaf litter
 720 mixtures. *Oikos* 78:527-538
 721 Mikutta R, Mikutta C, Kalbitz K, Scheel T, Kaiser K, Jahn R (2007) Biodegradation of forest floor
 722 organic matter bound to minerals via different binding mechanisms. *Geochim Cosmochim Acta*
 723 71:2569-2590
 724 Mitchell PJ, Simpson AJ, Soong R, Simpson MJ (2016) Biochar amendment altered the molecular-level
 725 composition of native soil organic matter in a temperate forest soil. *Environ Chem*:in press
 726 Mitchell PJ, Simpson AJ, Soong R, Simpson MJ (2015) Shifts in microbial community and water-
 727 extractable organic matter composition with biochar amendment in a temperate forest soil. *Soil*
 728 *Biol Biochem* 81:244-254
 729 Moore JD, Ouimet R (2014) Effects of two types of Ca fertilizer on sugar maple nutrition, vigor and
 730 growth after 7 years. *For Ecol Manage* 320:1-5

731 Moore JD, Ouimet R, Long RP, Bukaveckas PA (2015) Ecological benefits and risks arising from
 732 liming sugar maple dominated forests in northeastern North America. *Environ Rev* 23:66-77
 733 Mukherjee A, Zimmerman AR (2013) Organic carbon and nutrient release from a range of laboratory-
 734 produced biochars and biochar-soil mixtures. *Geoderma* 193:122-130
 735 Nelissen V, Rütting T, Huygens D, Staelens J, Ruyschaert G, Boeckx P (2012) Maize biochars
 736 accelerate short-term soil nitrogen dynamics in a loamy sand soil. *Soil Biol Biochem* 55:20-27
 737 Noyce GL, Basiliko N, Fulthorpe R, Sackett TE, Thomas SC (2015) Soil microbial responses over 2
 738 years following biochar addition to a north temperate forest. *Biol Fertil Soils* 51:649-659
 739 Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by
 740 extraction with sodium bicarbonate. *USDA Circular* 939:1-19
 741 Orth AB, Royse DJ, Tien M (1993) Ubiquity of lignin-degrading peroxidases among various wood-
 742 degrading fungi. *Appl Environ Microbiol* 59:4017-4023
 743 Otto A, Simpson MJ (2005) Degradation and preservation of vascular plant-derived biomarkers in
 744 grassland and forest soils from Western Canada. *Biogeochemistry* 74:377-409
 745 Otto A, Simpson MJ (2006a) Evaluation of CuO oxidation parameters for determining the source and
 746 stage of lignin degradation in soil. *Biogeochemistry* 80:121-142
 747 Otto A, Simpson MJ (2006b) Sources and composition of hydrolysable aliphatic lipids and phenols in
 748 soils from western Canada. *Org Geochem* 37:385-407
 749 Peng Y, Thomas SC (2010) Influence of non-nitrogenous soil amendments on soil CO₂ efflux and fine
 750 root production in an N-saturated northern hardwood forest. *Ecosystems* 13:1145-1156
 751 Prayogo C, Jones JE, Baeyens J, Bending GD (2014) Impact of biochar on mineralisation of C and N
 752 from soil and willow litter and its relationship with microbial community biomass and structure.
 753 *Biol Fertil Soils* 50:695-702
 754 Sackett TE, Basiliko N, Noyce GL, Winsborough C, Schurman J, Ikeda C, Thomas SC (2015) Soil and
 755 greenhouse gas responses to biochar additions in a temperate hardwood forest. *GCB Bioenergy*
 756 7:1062-1074
 757 Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in temperate
 758 forest soils. *Soil Biol Biochem* 51:115-124
 759 Sheibani S, Yanni SF, Wilhelm R, Whalen JK, Whyte LG, Greer CW, Madramootoo CA (2013) Soil
 760 bacteria and archaea found in long-term corn (*Zea mays* L.) agroecosystems in Quebec, Canada.
 761 *Can J Soil Sci* 93:45-57
 762 Simpson AJ, Song G, Smith E, Lam B, Novotny EH, Hayes MHB (2007) Unraveling the structural
 763 components of soil humin by use of solution-state nuclear magnetic resonance spectroscopy.
 764 *Environ Sci Technol* 41:876-883
 765 Simpson AJ, McNally DJ, Simpson MJ (2011) NMR spectroscopy in environmental research: from
 766 molecular interactions to global processes. *Prog Nucl Magn Reson Spectrosc* 58:97-175
 767 Singh N, Abiven S, Maestrini B, Bird JA, Torn MS, Schmidt MWI (2014) Transformation and
 768 stabilization of pyrogenic organic matter in a temperate forest field experiment. *Global Change*
 769 *Biol* 20:1629-1642
 770 Sitaula BK, Bakken LR, Abrahamsen G (1995) N-fertilization and soil acidification effects on N₂O and
 771 CO₂ emission from temperate pine forest soil. *Soil Biol Biochem*
 772 Sjöström E (1993) *Wood Chemistry, Fundamentals and Applications*, 2nd ed. Academic Press, London,
 773 UK
 774 Smebye A, Alling V, Vogt RD, Gadmar TC, Mulder J, Cornelissen G, Hale SE (2016) Biochar
 775 amendment to soil changes dissolved organic matter content and composition. *Chemosphere*
 776 142:100-105
 777 Smith JL, Collins HP, Bailey VL (2010) The effect of young biochar on soil respiration. *Soil Biol*
 778 *Biochem* 42:2345-2347

779 Soil Classification Working Group (1998) The Canadian System of Soil Classification. NRC Research
780 Press, Ottawa, ON, Canada

781 Spokas KA, Novak JM, Stewart CE, Cantrell KB, Uchimiya M, DuSaire MG, Ro KS (2011) Qualitative
782 analysis of volatile organic compounds on biochar. *Chemosphere* 85:869-882

783 Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and
784 soil microbial activity. *Soil Biol Biochem* 41:1301-1310

785 Sullivan BW, Alvarez-Clare S, Castle SC, Porder S, Reed SC, Schreeg L, Townsend AR, Cleveland CC
786 (2014) Assessing nutrient limitation in complex forested ecosystems: Alternatives to large-scale
787 fertilization experiments. *Ecology* 95:668-681

788 Thies JE, Rillig MC (2009) Characteristics of biochar: Biological properties. In: Lehmann J, Joseph S
789 (eds) *Biochar for Environmental Management: Science and Technology*. Earthscan, London, UK,
790 pp 85-105

791 Thomas SC, Gale N (2015) Biochar and forest restoration: a review and meta-analysis of tree growth
792 responses. *New For* 46:931-946

793 Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost
794 environment: A review. *Bioresour Technol* 72:169-183

795 Uchimiya M, Lima IM, Klasson KT, Wartelle LH (2010) Contaminant immobilization and nutrient
796 release by biochar soil amendment: Roles of natural organic matter. *Chemosphere* 80:935-940

797 van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: Soil microbes as
798 drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296-310

799 Vet R, Ro C (2008) Contribution of Canada-United States transboundary transport to wet deposition of
800 sulphur and nitrogen oxides - A mass balance approach. *Atmos Environ* 42:2518-2529

801 von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H
802 (2006) Stabilization of organic matter in temperate soils: Mechanisms and their relevance under
803 different soil conditions - A review. *Eur J Soil Sci* 57:426-445

804 Watzinger A, Feichtmair S, Kitzler B, Zehetner F, Kloss S, Wimmer B, Zechmeister-Boltenstern S, Soja
805 G (2014) Soil microbial communities responded to biochar application in temperate soils and
806 slowly metabolized ¹³C-labelled biochar as revealed by ¹³C PLFA analyses: Results from a short-
807 term incubation and pot experiment. *Eur J Soil Sci* 65:40-51

808 West TO, McBride AC (2005) The contribution of agricultural lime to carbon dioxide emissions in the
809 United States: Dissolution, transport, and net emissions. *Agric Ecosyst Environ* 108:145-154

810 Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of
811 microbial communities in soil: A review. *Biol Fertil Soils* 29:111-129

812 Zhu F, Lu X, Liu L, Mo J (2015) Phosphate addition enhanced soil inorganic nutrients to a large extent
813 in three tropical forests. *Sci Rep* 5:7923

814 Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization priming effects
815 among a variety of biochar-amended soils. *Soil Biol Biochem* 43:1169-1179

Table 1. Summary of microbial community and soil organic matter (OM) components that were characterized in the amended surface mineral soils (0-5 cm depth) using biomarker and NMR techniques, along with the results observed and their biogeochemical interpretation.

Measured soil biomarker	Information obtained	Results	Biogeochemical interpretation
<i>Phospholipid fatty acids (PLFAs)</i>			
• Bacterial PLFAs (Gram-positive, Gram-negative, actinomycetes)	• Soil bacterial activity	• Increased concentrations with biochar, P and biochar + P	• Bacterial activity was stimulated
• Fungal PLFA	• Soil fungal activity	• Increased concentrations with biochar and biochar + P • Concentration did not change with P fertilization	• Fungal activity was stimulated • No changes in fungal activity
• Ratio of Gram-negative/Gram-positive PLFAs	• Relative proportion of Gram-negative and Gram-positive bacteria in soil	• Ratio decreased with biochar, P and biochar + P	• Bacterial community shifted toward Gram-positive bacteria
• Ratio of bacterial/fungal PLFAs	• Relative proportion of bacteria and fungi in soil	• Ratio decreased with biochar and biochar + P • Ratio did not change with P fertilization	• Microbial community shifted toward fungi • Microbial community composition did not shift
<i>Solvent extraction</i>			
• Sugars	• Plant- and microbial-derived soil OM inputs	• Increased concentrations with biochar, P and biochar + P	• Higher inputs of plant- and microbial-derived OM
• Ergosterol	• Soil fungal activity	• Increased concentrations with biochar and biochar + P • Concentration did not change with P fertilization	• Fungal activity was stimulated • No changes in fungal activity
• Short-chain aliphatic lipids	• Microbial-derived soil OM inputs	• Increased concentrations with biochar and biochar + P Concentration did not change with P fertilization	• Higher inputs of microbial-derived OM • No changes in microbial-derived soil OM inputs
• Long-chain aliphatic lipids	• Plant-derived soil OM inputs	• Increased concentrations with biochar, P and biochar + P	• Higher inputs of plant-derived OM

• Steroids and triterpenoids	• Plant-derived soil OM inputs	• Increased concentrations with biochar, P and biochar + P	• Higher inputs of plant-derived OM
• Ratio of acyclic/cyclic lipids	• Relative proportion of acyclic and cyclic lipids in soil	• Ratio increased with biochar, P and biochar + P	• OM compositional shift toward a higher proportion of cyclic lipids
• Ratio of sterols/sterones	• Indicator of sterol degradation	• Ratio did not change with biochar, P or biochar + P	• No change in sterol degradation

Base hydrolysis

• Total cutin monomers	• Cutin-derived soil OM inputs	• Increased concentrations with biochar, P and biochar + P	• Higher inputs of cutin-derived OM components
• Total suberin monomers	• Suberin-derived soil OM inputs	• Increased concentrations with biochar, P and biochar + P	• Higher inputs of suberin-derived OM components
• Ratio of suberin/cutin	• Relative proportion of suberin and cutin in soil	• Ratio decreased with biochar, P and biochar + P	• OM compositional shift toward more cutin components
• Ratios of ω -C ₁₆ / Σ C ₁₆ and ω -C ₁₈ / Σ C ₁₈ acids	• Indicators of cutin degradation	• Ratio did not change with biochar, P or biochar + P	• No change in cutin degradation
• Ratio of Σ mid-chain acids/total suberin and cutin components	• Indicator of suberin and cutin degradation	• Ratio did not change with biochar, P or biochar + P	• No change in suberin and cutin degradation

CuO oxidation

• Total lignin monomers and total lignin dimers	• Extractable monomeric and dimeric sidechains of lignin	• Increased concentrations with biochar and biochar + P
• Concentration did not change with P fertilization	• Higher concentrations of extractable lignin components
• No changes in extractable lignin
• Ratio of lignin monomers/dimers	• Relative proportion of extractable monomeric and dimeric lignin sidechains	• Ratio increased with biochar and biochar + P
• Ratio did not change with P fertilization	• Macromolecular lignin contained a greater proportion of monomeric sidechains
• No changes in the proportion of monomers and dimers

<ul style="list-style-type: none"> • Acid-to-aldehyde ratios (syringyl and vanillyl monomers) 	<ul style="list-style-type: none"> • Indicator of lignin sidechain oxidation 	<ul style="list-style-type: none"> • Ratios did not change with biochar, P or biochar + P 	<ul style="list-style-type: none"> • No change in lignin oxidation
<u>Solution-state 1H NMR spectroscopy</u>			
<ul style="list-style-type: none"> • Base-extractable soil OM 	<ul style="list-style-type: none"> • Relative proportion of specific organic C functional groups in soil OM 	<ul style="list-style-type: none"> • Decreased proportion of carbohydrates, increased proportion of alkyl-derived OM with biochar, P and biochar + P 	<ul style="list-style-type: none"> • Enhanced degradation of labile OM, accumulation of more recalcitrant alkyl OM components

Figure captions

Fig. 1 Principal component analysis (PCA) scores plots of (a) phospholipid fatty acid and (b) total soil organic matter biomarker concentrations in the biochar, P and biochar + P-amended soils at the 0-5 cm and 5-15 cm depths. The percentage of variance explained by each component is indicated in brackets on each axis. The values shown are mean scores with standard error ($n = 3$). Treatment data labels indicate the biochar application rate and the presence or absence of P fertilization

Fig. 2 Total concentrations of (a) Gram-negative, (b) Gram-positive, (c) actinomycete and (d) fungal phospholipid fatty acids (PLFAs) as well as the ratios of (e) Gram-negative/Gram-positive PLFAs and (f) bacterial/fungal PLFAs in the 0-5 cm mineral soil during a three-year biochar and P amendment study. Treatment data labels indicate the biochar application rate and the presence or absence of P fertilization. Asterisks indicate values that are statistically significant ($p \leq 0.05$) from the unamended soil

Fig. 3 Total concentrations of solvent-extractable (a) sugars, (b) short-chain *n*-alkanoic acids, (c) long-chain *n*-alkanes, (d) long-chain *n*-alkanols, (e) long-chain *n*-alkanoic acids, (f) steroids, (g) triterpenoids and (h) ergosterol as well as (i) the ratio of acyclic/cyclic aliphatic lipids in the 0-5 cm mineral soil during a three-year biochar and P amendment study. Treatment data labels indicate the biochar application rate and the presence or absence of P fertilization. Asterisks indicate values that are statistically significant ($p \leq 0.05$) from the unamended soil

Fig. 4 Total concentrations and ratios of biomarkers isolated using chemolytic extraction techniques, including base-hydrolyzable (a) cutin and (b) suberin compounds, (c) the ratio of suberin/cutin biomarkers, as well as CuO oxidation products including lignin-derived phenol (d) monomers, (e)

dimers and (f) the ratio of monomers/dimers in the 0-5 cm mineral soil during a three-year biochar and P amendment study. Treatment data labels indicate the biochar application rate and the presence or absence of P fertilization. Asterisks indicate values that are statistically significant ($p \leq 0.05$) from the unamended soil

Fig. 5 Representative solution-state (a) 1-D ^1H , (b) 1-D diffusion-edited ^1H and (c) 2-D ^1H - ^{13}C heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectra of base-extractable soil OM in the amended soil samples. Braces indicate integrated regions of the 1-D spectra for protons in the following specific chemical environments: (1) aliphatic (0.6-1.3 ppm); (2) *N*- and *O*-substituted aliphatic (1.3-2.9 ppm); (3) *O*-alkyl (2.9-4.1 ppm); (4) α -proton of peptides (4.1-4.8 ppm); (5) anomeric protons of carbohydrates (4.8-5.2 ppm); (6) aromatic and phenolic (6.2-7.8 ppm); and (7) amide (7.8-8.4 ppm). The asterisk (*) denotes the NMR solvent ($\text{DMSO-}d_6$). The arrows and dashed boxes outline regions of the 2-D ^1H - ^{13}C HSQC NMR spectrum that were used to calculate normalized area ratios (defined in Table S19 of the Supplementary Material)

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5