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1 **Effects of biochar on carbon and nitrogen fluxes in boreal forest soil**

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22

23 **Abstract**

24

25 *Background and aims* The addition of biochar to soil may offer a chance to mitigate climate change  
26 by increasing soil carbon stocks, improving soil fertility and enhancing plant growth. The impacts of  
27 biochar in cold environments with limited microbial activity are still poorly known.

28 *Methods* In order to understand to what extent different types and application rates of biochar affect  
29 carbon (C) and nitrogen (N) fluxes in boreal forests, we conducted a field experiment where two  
30 different spruce biochars (pyrolysis temperatures 500°C and 650°C) were applied at the rate of 0, 5  
31 and 10 t ha<sup>-1</sup> to *Pinus sylvestris* forests in Finland.

32 *Results* During the second summer after treatment, soil CO<sub>2</sub> effluxes showed no clear response to  
33 biochar addition. Only in June, the 10 t ha<sup>-1</sup> biochar (650°C) plots had significantly higher CO<sub>2</sub>  
34 effluxes compared to the control plots. The pyrolysis temperature of biochar did not affect soil CO<sub>2</sub>  
35 effluxes. Soil pH increased in the plots receiving 10 t ha<sup>-1</sup> biochar additions. Biochar treatments had  
36 no significant effect on soil microbial biomass and biological N fixation. Nitrogen mineralization  
37 rates in the organic layer tended to increase with the amount of biochar, but no statistically significant  
38 effect was detected.

39 *Conclusions* The results suggest that wood biochar amendment rates of 5–10 t ha<sup>-1</sup> to boreal forest  
40 soil do not cause large or long-term changes in soil CO<sub>2</sub> effluxes or reduction in native soil C stocks.  
41 Furthermore, the results imply that biochar does not adversely affect soil microbial biomass or key N  
42 cycling processes in boreal xeric forests, at least within this time frame. Thus, it seems that biochar  
43 is a promising tool to mitigate climate change and sequester additional C in boreal forest soils.

44

45 **Key words:** Biochar; biological nitrogen fixation; microbial biomass; nitrogen mineralization;  
46 nitrification; soil respiration

## 47 **Introduction**

48

49 Biochar is formed by heating organic material under low oxygen concentrations in a process known  
50 as pyrolysis (Lehmann and Joseph 2012). The addition of biochar to soil is a potential tool for carbon  
51 (C) sequestration and climate change mitigation because biochar is enriched in C and recalcitrant to  
52 decomposition in comparison to the original biomass (Woolf et al. 2010; Gurwick et al. 2013).  
53 Biochar can also act as a soil conditioner enhancing plant growth by increasing soil microbial activity,  
54 water holding capacity, cation exchange capacity and pH (Lehmann and Joseph 2012; Robertson et  
55 al. 2012; Biederman and Harpole 2013; Thomas and Gale 2015). However, these changes in soil  
56 chemical and physical properties may increase microbial biomass, microbial activity and the  
57 decomposition of soil organic matter (Lehmann and Joseph 2012). Moreover, the labile C fractions  
58 of biochar may accelerate the decomposition of old soil organic matter through the priming effect  
59 (Cross and Sohi 2011; Zimmerman et al. 2011; Fang et al. 2015; Wang et al. 2015). In addition,  
60 biochar may affect the chemistry of phenolic compounds which commonly inhibit the decomposition  
61 of soil organic matter in boreal forest soils. Fire-derived charcoal have been found to adsorb phenolic  
62 compounds and to accelerate organic matter decomposition in boreal forests (Zackrisson et al. 1996;  
63 Wardle et al. 1998, 2008). Accelerated decomposition of native soil C increases soil CO<sub>2</sub> emissions  
64 and reduces the soil C stocks, which is contradicting the idea of C sequestration.

65

66 The impacts of biochar addition on soil processes have been variable and are dependent on the  
67 pyrolysis temperature and the feedstock of biochar (Spokas and Reicosky 2009; Ameloot et al. 2013;  
68 Biederman and Harpole 2013; Lei and Zhang 2013; Stewart et al. 2013) soil properties (Kolb et al.  
69 2009; Spokas and Reicosky 2009), vegetation and local environmental and climatic conditions (He  
70 et al. 2017). Previous studies have mainly been conducted on agricultural soils in tropical and  
71 temperate regions, and very little information exists about the stability of biochar in the soil and the

72 effects of biochar additions on C and nutrient cycling in forests, especially in the boreal zone (Liu et  
73 al. 2015; Bruckman et al. 2016). The use of forest biomass as an energy source has increased in  
74 Europe (Helmisaari et al. 2014). Instead of traditional burning, part of the forest biomass could be  
75 converted to biochar, which can be incorporated back into soil, where it helps to improve the  
76 sustainability of bioenergy harvesting if part of C and nutrients were recycled back to the forests and  
77 if biochar acts as a soil amendment.

78

79 In boreal forests, most of the soil nitrogen (N) is in organic form, N mineralization rates are low and  
80 tree growth is N-limited (Sponseller et al. 2016). The mineralization of N can be accelerated if biochar  
81 stimulates soil organic matter decomposition which, in turn, may have a positive feedback on  
82 ecosystem net primary production and CO<sub>2</sub> fixation. Biochar application has been shown to increase  
83 net N mineralization and nitrification rates (Ameloot et al. 2015; Case et al. 2015; Gundale et al.  
84 2015) which has been attributed to increased soil pH, enhanced microbial growth and activity and the  
85 sorption of phenols and terpenes onto biochar (Clough and Condron 2010; Lehmann et al. 2011).  
86 Polyphenolics and terpenes inhibit nitrification and net N mineralization by decreasing the activity of  
87 enzymes involved in N cycling (Adamczyk et al. 2015, 2017). Wildfire-produced charcoal has been  
88 found to adsorb phenols, and to increase net N mineralization and nitrification in forest soils  
89 (Zackrisson et al. 1996; Wardle et al. 1998; DeLuca et al. 2006; Ball et al. 2010). Biochar may thus  
90 serve as an important soil amendment, and it could be possibly used for mimicking the effects of fire-  
91 derived charcoal in Finland, where forest fires are effectively controlled (total area of forest fires is  
92 only 300-1000 ha<sup>-1</sup> yr<sup>-1</sup>) and forest soils contain high amounts of phenolic compounds. On the other  
93 hand, the reduction of N mineralization and increased N immobilization may occur when biochar  
94 compounds with a high C:N ratio are microbially degraded (Bruun et al. 2012; Dempster et al. 2012;  
95 Prommer et al. 2014) and due to the adsorption of NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup> onto the biochar surface (Clough  
96 and Condron 2010).

97

98 Many boreal forests receive low amounts of N deposition and biological N fixation contributes  
99 significantly to N input in these ecosystems (Granhall and Lindberg 1980; DeLuca et al. 2002;  
100 Sponseller et al. 2016). Feather mosses that support epiphytic cyanobacteria represent the primary  
101 source of biological N-fixation in boreal coniferous forests (Zackrisson et al. 2004), but there are also  
102 free-living N-fixing bacteria in forest soils (Granhall and Lindberg 1980; Limmer and Drake 1996).  
103 The influence of biochar amendment on N-fixation in boreal forests is not yet known. Biochar may  
104 affect the magnitude of biological N-fixation by changing the biomass and species composition of  
105 mosses (Zackrisson et al. 2004). Increased soil pH and more favourable soil moisture conditions after  
106 biochar addition may enhance N-fixation (Nohrstedt 1985; Limmer and Drake 1996) whereas  
107 increased availability of inorganic N may have a suppressing effect (Zackrisson et al. 2004; DeLuca  
108 et al. 2007).

109

110 The purpose of our study was to determine whether biochar additions increase soil pH, soil microbial  
111 biomass and N transformations (net N mineralization, ammonification and nitrification) in boreal  
112 forest soil. Additionally, we examined whether biochar affects soil CO<sub>2</sub> fluxes and biological N-  
113 fixation rates. We hypothesized that biochar amendment will increase soil pH and microbial biomass,  
114 resulting in increased soil respiration, N-mineralization, nitrification and N-fixation. We also  
115 hypothesize that these increases will occur to a greater extent at higher biochar amounts. The effects  
116 of biochar on soil C and N fluxes were studied in the second year after the treatment. Generally  
117 biochar causes at least a short-term limited positive priming effect (Bruckman et al. 2015; Mitchell  
118 et al. 2015; Page-Dumroese et al. 2017), but the longer-term field experiments about the impacts of  
119 biochar in forest ecosystems are rare. Biochar increased soil respiration in our study plots during the  
120 first months after treatment (Palviainen et al. 2017a), and we wanted to know whether biochar  
121 addition alters soil CO<sub>2</sub> effluxes for a longer term in boreal forest soil.

122

## 123 **Materials and methods**

124

### 125 Study area

126

127 The study area situates in southern Finland in Juupajoki (61° 48' N, 24° 18' E, 181 m a.s.l.) close to  
128 Hyytiälä Forestry Field Station. The experiment was performed in young ~20-year-old Scots pine  
129 (*Pinus sylvestris* L.) forest stands that were naturally regenerated from seed trees after clear-cutting.  
130 The sites were nutrient poor xeric (*Calluna*) and sub-xeric (*Vaccinium*) forest site types (Cajander  
131 1949). The mean height of trees was 5.0 m, diameter at breast height (1.3 m) was 4.9 cm, and the  
132 number of trees (height > 1.3 m) was 4025 ha<sup>-1</sup>. Understory vegetation is dominated by dwarf shrubs  
133 (*Vaccinium vitis-idaea* L., *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L. and *Vaccinium myrtillus*  
134 L.), mosses (*Pleurozium schreberi* (Brid.) Mitt. and *Dicranum polysetum*) and lichens (*Cladina* sp.).  
135 The terrain is flat and the soil is a nutrient-poor, well-drained haplic podzol (IUSS Working Group  
136 WRB, FAO 2015). The soil texture is coarse sand. The long-term (1981–2010) mean annual  
137 temperature in the area is 3.5°C and annual precipitation is 700 mm (Pirinen et al. 2012). During the  
138 experimental period in summer 2016, mean air temperature was 14.0°C in June and 16.0°C in July.  
139 Precipitation was 124 mm in June and 119 mm in July in the year 2016.

140

141 The experiment was set up as a replicated split plot experiment with four replicates (called whole  
142 plots) and five subplots (15 m × 15 m) within each whole plot. Whole plots were separated by a few  
143 hundred meters from each other and belonged to different forest stands to avoid pseudo-replication.  
144 The subplots were amended with biochar produced from Norway spruce (*Picea abies* (L.) H. Karst)  
145 wood chips at two different temperatures, at 500°C and at 650°C (manufactured by Sonnenerde  
146 GmbH, Riedlingsdorf, Austria). The biochar was produced by using the Pyreg process and the grain

147 size was 5-10 mm (Bruckman et al. 2015, Fig. 1). Both types of biochar were applied on the plots at  
148 two different amounts, 0.5 kg m<sup>-2</sup> and 1.0 kg m<sup>-2</sup>. Thus, in each whole plot there were five treatments:  
149 a control without biochar, 500°C biochar 0.5 kg m<sup>-2</sup>, 500°C biochar 1.0 kg m<sup>-2</sup>, 650°C biochar 0.5 kg  
150 m<sup>-2</sup> and 650°C biochar 1.0 kg m<sup>-2</sup>. There was a 10-meter buffer zone between each subplot. Biochar  
151 was spread manually on the top of the organic layer during the last two weeks of May in 2015 (Fig.  
152 1). Biochar was spread to the soil surface to avoid soil disturbance and damage to roots. The amounts  
153 of biochar correspond to 5 and 10 t ha<sup>-1</sup>, which are typical and economically feasible biochar  
154 application rates in forests (Bruckman et al. 2016). The added amounts of biochars were considerably  
155 higher than the amounts of charcoal, or black C (range 0-2220 kg ha<sup>-1</sup>, mean 770 kg ha<sup>-1</sup>) originated  
156 by forest fires in Scandinavian boreal forests (Ohlson et al. 2009).

157

#### 158 Soil and biochar analyses

159

160 Soil samples were collected from the organic layer and the upper 15 cm mineral soil layer using  
161 stainless soil corer (diameter 5.5 cm) at nine locations in each subplot in mid-May in 2015 just before  
162 biochar addition. The samples were dried (60°C, 24 h), sieved through a 2-mm sieve, and ground  
163 before the analysis. Subsamples were taken for dry mass determination at 105°C. Soil particle size  
164 distribution was determined by the laser diffraction (LS230, Coulter Corp., Miami, Florida, USA)  
165 method (Table 1). The C and N concentrations of soil and biochars were analyzed with an elemental  
166 analyzer (Vario Max CN elemental analyser, Elementar Analysensysteme GmbH, Germany). The  
167 loss on ignition (LOI) of biochars were determined by combusting samples at 550°C for 3 hours. The  
168 concentrations of P, K, Ca, Mg, S, Fe, Al, Na, Cu, Mn, Ni, Si and Zn in biochar were determined  
169 from HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> digestion by ICP atomic emission spectrophotometer (ARL 3580 OES, Fison  
170 Instruments, Valencia, USA). Biochar pH was determined using a pH meter (PHM210, Radiometer  
171 Analytical, France) on a 1:2.5 (v:v) biochar /water solution and electric conductivity was measured



172 by an electric meter (JENWAY 4010 Conductivity, TER Calibration Ltd., Wigan, UK). The  
173 properties of biochars are presented in Table 2.

174

175 Soil temperature and soil respiration measurements

176

177 Soil temperature was measured continuously on all sample plots at three hours intervals with iButton  
178 temperature sensors (Maxim Integrated, San Jose, California, U.S.A.), that were installed under the  
179 organic layer. We interpolated hourly values from which we calculated daily mean temperatures for  
180 each plot.

181

182 Six polyvinyl chloride (PVC) collars (diameter 0.22 m) were installed permanently into the soil in  
183 each of the 20 subplots in the summer of 2015 for soil respiration measurements. Thus, there were 24  
184 collars in each treatment and 120 collars in total. The lower edge of the collar was placed at 0.02 m  
185 depth in the mor layer above the rooting zone to avoid damaging the roots. The collars were sealed  
186 with a thin layer of sand placed around the collar. Ground vegetation inside the collars remained  
187 intact.

188

189 Soil respiration i.e. CO<sub>2</sub> effluxes were measured with a closed chamber system consisting of an  
190 opaque cylindrical polycarbonate chamber (diameter 20 cm, height 30 cm), a CO<sub>2</sub> analyzer, sensor  
191 for relative humidity and temperature and a data logger (Kulmala et al. 2008; Pumpanen et al. 2015).  
192 The CO<sub>2</sub> concentration inside the chamber was recorded with a GMP343 diffusion type CO<sub>2</sub> probe  
193 (Vaisala Oy, Vantaa, Finland) at 5-second intervals and corrected automatically for humidity,  
194 temperature and pressure with a data recorder (MI70, Vaisala Oyj) using the readings from the  
195 temperature and humidity probe (HMP75, Vaisala Oyj) inside the chamber. Air pressure was

196 measured daily at the nearby SMEAR II station (4 km away). During the measurements, air inside  
197 the chamber was mixed continuously by a small fan.

198

199 The chamber was placed onto the collars only during the measurements which lasted 4 minutes. Soil  
200 respiration measurements were conducted with two chambers in two consecutive days in June and  
201 July 2016 (i.e. 13 and 14 months after biochar addition). All collars were measured before noon to  
202 minimize daily temperature fluctuations. Air temperature during the soil respiration measurements  
203 varied  $\pm 0.7^{\circ}\text{C}$  in June and  $\pm 1.0^{\circ}\text{C}$  in July, and the variation in soil temperatures was even smaller  
204 ( $\pm 0.3^{\circ}\text{C}$ ) indicating that temperature fluctuations during the measurements did not markedly affect  
205 the results. Headspace volume was corrected for the varying height of the collars. Soil temperature at  
206 5 cm depth was measured by a dual input digital thermometer (Fluke-52-2, Fluke Corp.)  
207 simultaneously near the collar. The  $\text{CO}_2$  efflux was calculated as the slope of a linear regression of  
208  $\text{CO}_2$  concentration in the chamber against time. Only measurements taken between 45 seconds and 3  
209 minutes after the closure were included in the fitting.

210

211 Nitrogen mineralization experiment

212

213 Nine soil core samples (diameter 5.5 cm) were collected in November 2016 from the organic layer  
214 and the upper 10 cm mineral soil layer from the control subplots and from the subplots where  $650^{\circ}\text{C}$   
215 produced biochar were added  $5 \text{ t ha}^{-1}$  and  $10 \text{ t ha}^{-1}$ , respectively. Soil samples were stored at  $+5^{\circ}\text{C}$  in  
216 plastic bags for a few days before further treatment. The nine soil samples from each subplot were  
217 combined to give three composite samples per subplot ( $n= 12/\text{treatment}$ ). To homogenize the soil  
218 material, the samples were sieved through a 2-mm sieve. Nitrogen transformations were studied by  
219 incubating 10 g of humus and 20 g of mineral soil in cork sealed 125-ml glass bottles in a climate  
220 chamber (WEISS WK11 340, Weiss Klimatechnik GmbH, Germany) at constant temperature ( $15^{\circ}\text{C}$ )

221 and moisture (soil moisture content adjusted to 60% of the water-holding capacity) for 42 days. At  
222 the start and at the end of the incubation, an analysis of inorganic N was performed to estimate net N  
223 mineralization, ammonification and nitrification for the samples. Each soil sample was extracted with  
224 40 ml of 1 M KCl for 2 h (ISO 14256-2: 2005). The KCl extracts were filtered through a 0.45- $\mu$ m  
225 filter and ammonium ( $\text{NH}_4\text{-N}$ ) and nitrate ( $\text{NO}_3\text{-N}$ ) concentrations were analyzed with a flow-  
226 injection ion analyzer (Lachat Quickchem 8000, Milwaukee, WI, USA). Initial concentrations of  
227 ( $\text{NH}_4^+\text{-N}$ ) and ( $\text{NO}_3^-\text{-N}$ ) were subtracted from the corresponding post-incubation concentrations to  
228 calculate the rates of net ammonification and nitrification. Net mineralized N was calculated from the  
229 sum of ( $\text{NH}_4^+\text{-N}$ ) and ( $\text{NO}_3^-\text{-N}$ ) accumulated during the period of incubation. The incubated soil  
230 samples were dried, ground with a mortar grinder (Retsch RMO Mortar Grinder, Retsch GmbH,  
231 Germany) and their C and N concentrations were measured with an elemental analyser (Vario Max  
232 CN, Elementar Analysensysteme GmbH, Germany). A subsample was taken for dry mass  
233 determination (105°C, 24 h). The formed inorganic N was expressed on organic matter basis ( $\mu\text{g N}$ ,  
234  $\text{NO}_3$  or  $\text{NH}_4$   $\text{g C}^{-1} \text{d}^{-1}$ ).

235

236 Soil pH was measured from separate samples by mixing 10 ml of soil with 25 ml of deionized water.  
237 The suspension pH ( $\text{H}_2\text{O}$ ) was measured with a glass electrode (PHM210, Radiometer Analytical,  
238 France) after 24 hours.

239

240 Biological nitrogen fixation and moss biomass

241

242 The samples containing mosses and organic layer were collected in May, June and July 2016 with a  
243 soil core cylinder (diameter 5.8 cm) from the control subplots and from the subplots where 650°C  
244 produced biochar were added 5  $\text{t ha}^{-1}$  and 10  $\text{t ha}^{-1}$ , respectively. In total, 108 samples were collected  
245 for biological N fixation measurements (12 samples per treatment, 3 treatments and 3 sampling

246 times). Biological N fixation was estimated using acetylene reduction method (Hardy et al. 1968).  
247 The samples included organic layer because in boreal forests N-fixation occurs both in the organic  
248 layer and mosses (Granhall and Lindberg 1980; Limmer and Drake 1996). The whole samples were  
249 placed in 500 ml glass jars with rubber septum caps, after which 10% of the volume of the jar was  
250 evacuated using a gas-tight syringe (BD Plastipak 60, BOC Ohmeda, Helsingborg, Sweden) and  
251 replaced with acetylene. The samples were incubated in an environmental chamber (WEISS WK11  
252 340, Weiss Klimatechnik GmbH, Germany) with artificial light (LED Grow Light Spider 1) at 10°C  
253 (samples collected in May), 15°C (samples collected in June) and 20°C (samples collected in July)  
254 for 24 hours. After incubation, a gas sample was taken from each jar by a 50-ml polypropylene syringe  
255 (BD Plastipak 60, BOC Ohmeda, Helsingborg, Sweden), injected into a 12 ml exetainer vial (Labco  
256 limited, Lampeter, UK) and the ethylene concentrations were analysed with a gas chromatograph  
257 (HP6890) with flame ionization detector as described before (Leppänen et al. 2013). A commonly  
258 used ratio of 3 moles of reduced acetylene per mole of N fixed was used to calculate the mass of fixed  
259 N (DeLuca et al. 2002). The biomass of different moss species was determined after drying the  
260 samples at 60°C for 48 hours to see whether the biochar amendment affects the biomass of mosses,  
261 and to explain possible differences in N-fixation rates between treatments.

262

#### 263 Soil microbial biomass

264

265 Twelve soil core (diameter 10.0 cm) samples per treatment were collected for microbial biomass C  
266 and N analysis both in June and July of 2016 from the organic layer from the control subplots and  
267 from the subplots where 650°C produced biochar was added. Root material was removed with  
268 tweezers, the samples were placed into 45 ml plastic tubes and stored in the freezer at -20°C. The  
269 samples were kept 7–10 days at + 5 °C before analysis. Samples were sieved through a 2-mm sieve,  
270 grinded (DeLonghi KG49) and a subsample was taken for dry mass determination (105 °C, 24 h).

271 Soil microbial biomass C and N were determined by a chloroform fumigation extraction method  
272 (Brookes et al. 1985; Vance et al. 1987). Three grams of soil from each sample was weighed, placed  
273 into glass beakers and fumigated with 30 ml ethanol-free chloroform (CHCl<sub>3</sub>) in a vacuum desiccator.  
274 Another equivalent sample weighting three grams was placed in plastic bottles in another desiccator  
275 as un-fumigated control samples. Both desiccators were kept at 25 °C in the dark for 24 hours. After  
276 fumigation, 0.5 M potassium sulfate (K<sub>2</sub>SO<sub>4</sub>) (with the ratio of oven-dry basis soil: K<sub>2</sub>SO<sub>4</sub>=1:20) was  
277 used to extract the fumigated and un-fumigated samples. Then the samples were shaken at 200 rpm  
278 for 1 hour and filtered using Whatman No.42 ashless filter papers. The filtrate was then used to  
279 analyze the microbial C and N by a TOC-VCPH analyzer (Shimadzu Corp., Kyoto, Japan). Microbial  
280 biomass C and N were calculated as the difference between fumigated and unfumigated samples and  
281 the difference was divided by the soil-specific calibration factor which was 0.45 for C (Beck et al.  
282 1997) and 0.54 for N (Brookes et al. 1985).

283

#### 284 Statistical analyses

285

286 The effect of biochar amendment on soil pH, soil temperature, soil respiration, soil microbial biomass,  
287 biological N fixation and N mineralization were analyzed with linear mixed model followed by  
288 Fisher's least significant difference (LSD) test. Treatment was a fixed factor and plot was a random  
289 factor. In the soil respiration analyses, the collar within the subplot was set as random factor. Data  
290 were checked for normality with the Shapiro–Wilk test and the recorded CO<sub>2</sub> effluxes were  
291 logarithm-transformed. Differences were considered statistically significant when P was ≤ 0.05.  
292 Statistical tests were performed using IBM SPSS version 23 (IBM Corp, Armonk, NY, USA). The  
293 results of the statistical tests are presented in supplementary material.

294

## 295 **Results**

296

## 297 Biochar characteristics

298

299 Carbon concentrations were similar in both biochars, while the concentrations of N and other  
300 macronutrients tended to be lower in 650°C than in 500°C biochar (Table 2). Also C:N ratio was  
301 considerably higher in 650°C than 500°C biochar. Altogether, 3031 kg ha<sup>-1</sup> and 6061 kg ha<sup>-1</sup> of C  
302 were added to the soil along with 5 t and 10 t ha<sup>-1</sup> biochar treatments, respectively. These amounts  
303 correspond to 14% and 28% of soil C pools (organic layer and 0–15 cm mineral soil layer) in the  
304 study site (Table 1).

305

## 306 Soil temperature and soil respiration

307

308 Soil temperatures did not differ significantly among the treatments (Table 3). Treatment had  
309 significant effect on soil respiration in June (F= 3.978, P= 0.005) but not in July (F= 1.411, P=0.259).  
310 Soil temperature as a covariate was not significant (June: F=0.852, P= 0.358, July: F=0.695, P=0.407)  
311 and inclusion of this covariate in the analysis did not affect the results. In June, soil CO<sub>2</sub> efflux was  
312 significantly higher in plots where 650°C produced biochar was applied 10 t ha<sup>-1</sup> compared to control  
313 and 5 t ha<sup>-1</sup> biochar treatments (Fig. 2). Both in June and July, 500°C biochar plots had higher soil  
314 CO<sub>2</sub> effluxes in 10 t ha<sup>-1</sup> treatments compared to 5 t ha<sup>-1</sup> treatments (Fig. 2). The production  
315 temperature of biochar did not have an effect on soil CO<sub>2</sub> fluxes, as there was no statistically  
316 significant difference in CO<sub>2</sub> effluxes between 500°C and 650°C biochar subplots in 5 t and 10 t ha<sup>-1</sup>  
317 treatments.

318

## 319 Soil microbial biomass, moss biomass and biological N fixation

320

321 The biochar treatments did not significantly influence soil microbial biomass C or soil microbial  
322 biomass N (Fig. 3). Microbial biomass C:N-ratio was significantly higher in 5 t ha<sup>-1</sup> biochar plots  
323 than in 10 t ha<sup>-1</sup> biochar plots in June, but microbial biomass C:N-ratios did not differ between  
324 treatments in July (Fig. 3).

325

326 The total biomass of mosses was similar between control and biochar treatments, but there were slight  
327 differences in species abundances because the biomass of *Pleurozium schreberi* was significantly  
328 higher in 5 t ha<sup>-1</sup> biochar plots than in 10 t ha<sup>-1</sup> biochar and control plots (Table 4).

329

330 Biochar amendment had no significant effect on biological N fixation rate (Fig. 4). Nitrogen fixation  
331 rates were significantly higher at an incubation temperature of 20°C (P <0.001) but did not differ  
332 between 10°C and 15°C. The mean N fixation rates were 199, 233 and 439 µg N m<sup>-2</sup> d<sup>-1</sup>, at 10°C,  
333 15°C and 20°C, respectively. By taking into account the average length of growing season (180 days)  
334 and mean air temperature (~15°C) during growing season in the study area, the measured N fixation  
335 rates correspond to 0.56, 0.43 and 0.58 kg ha<sup>-1</sup> yr<sup>-1</sup> in control, 5 t ha<sup>-1</sup> and 10 t ha<sup>-1</sup> biochar treatments,  
336 respectively.

337

338 Soil pH and N transformations

339

340 Soil pH in the organic layer and the upper 10 cm mineral soil layer was significantly higher (P <0.04)  
341 in 10 t ha<sup>-1</sup> treated biochar plots than in the control plots (Fig. 5). In the control plots, soil pH was 3.7  
342 in the organic layer and 4.1 in the upper 10 cm mineral soil, whereas in 10 t ha<sup>-1</sup> treated biochar plots  
343 the respective values were 4.1 and 4.3.

344

345 The biochar treatments did not induce statistically significant effects on net N mineralization,  
346 ammonification or nitrification rates (Fig. 6). The average net N mineralization rates in the organic  
347 layer increased with the amount of biochar, being 0.95, 2.30 and 2.78  $\mu\text{g N g C}^{-1} \text{ day}^{-1}$  in the control,  
348 5 t ha<sup>-1</sup> biochar plots and 10 t ha<sup>-1</sup> biochar plots, respectively. However, this difference was not  
349 statistically significant ( $P > 0.05$ ) due to the high variation within each treatment. In the mineral soil,  
350 net N mineralization was small or N was immobilized. Net nitrification was also negligible.

351

## 352 **Discussion**

353

354 Few studies have investigated *in-situ* the effects of biochar addition on soil respiration in forest  
355 ecosystems. There was no clear and consistent tendency towards increased soil CO<sub>2</sub> effluxes during  
356 the second summer after biochar addition. Only in June, the CO<sub>2</sub> effluxes were significantly (17%)  
357 higher in 10 t ha<sup>-1</sup> 650°C produced biochar plots than in the control plots. Otherwise, there were no  
358 differences in soil CO<sub>2</sub> effluxes between control and biochar treatments. Slightly increased soil CO<sub>2</sub>  
359 effluxes after biochar addition may be observed due to the mineralization of labile C fractions of  
360 biochar and/or biochar induced priming effects in the soil shortly after biochar amendment (Smith et  
361 al. 2010; Zimmerman 2011; Cross and Sohi 2011; Jones et al. 2011). Biochar may also indirectly  
362 stimulate microbial activity by providing nutrients, offering a habitat because of its porous structure,  
363 increasing soil pH and reducing the bioavailability of toxic compounds in soil through sorption  
364 (Steinbeiss et al. 2009; Lehmann et al. 2011; Lehmann and Joseph 2012; Hammer et al. 2014). In  
365 addition, biochar may increase plant growth and root biomass (Lehmann et al. 2011; Robertson et al.  
366 2012; Thomas and Gale 2015), which promotes root respiration and provides additional organic  
367 matter for decomposition.

368



369 Both increased and decreased C mineralization has been observed following biochar addition to  
370 various types of soils (Cross and Sohi 2011; Zimmerman et al. 2011; Liu et al. 2015; Wang et al.  
371 2015). Studies from temperate forests have reported short-term positive priming effects or unchanged  
372 soil respiration after biochar addition (Sackett et al. 2014; Bruckman et al. 2015). Gundale et al.  
373 (2015) mixed 10 t ha<sup>-1</sup> biochar to boreal forest soil and did not find significant effect on soil  
374 respiration. In general, the positive priming effects are observed in soils which have low C contents  
375 (Zimmerman et al. 2011). Weak priming effects and moderate changes in CO<sub>2</sub> effluxes in boreal  
376 forest soils after biochar addition may take place since boreal forest soils have high C content (DeLuca  
377 and Boisvenue 2012).

378

379 The responses of soil CO<sub>2</sub> effluxes depend also on feedstock characteristics, pyrolysis temperature  
380 and application rate (Zimmerman et al. 2011; He et al. 2017). In general, wood biochars increase soil  
381 CO<sub>2</sub> effluxes to a lesser degree compared to other types of biochars, and soil CO<sub>2</sub> effluxes decline  
382 with biochar pyrolysis temperature (Zimmerman et al. 2011; He et al. 2017). In the present study,  
383 wood biochar, produced at relatively high temperatures, may be the reason for the small changes in  
384 soil respiration. Furthermore, apparently moderate biochar amendments do not cause large increases  
385 in soil respiration. For example, meta-analyses from croplands have showed that biochar increases  
386 soil CO<sub>2</sub> emissions significantly only at high (20–40 t ha<sup>-1</sup>) amendment rates (Song et al. 2016; He et  
387 al. 2017). However, our results also showed that there was tendency for higher soil CO<sub>2</sub> effluxes from  
388 10 t ha<sup>-1</sup> plots than from 5 t ha<sup>-1</sup> plots, at least in 500°C biochar treatments (Fig. 2). Pyrolysis  
389 temperature did not have an effect on soil CO<sub>2</sub> fluxes, although generally biochars produced at high  
390 (> 600°C) temperatures are more recalcitrant than those produced at lower temperatures (Cross and  
391 Sohi 2011; Ameloot et al. 2013; Fang et al. 2015) and they often cause negative priming effects in  
392 the soil (Zimmerman et al. 2011; Song et al. 2016).

393

394 The differences in soil CO<sub>2</sub> efflux among our biochar and control plots were higher during the first  
395 summer (Palviainen et al. 2017a) when compared to the second summer. Soil CO<sub>2</sub> effluxes at 10 t ha<sup>-1</sup>  
396 biochar treatments (both 500°C and 650°C biochar treatments) were significantly higher compared  
397 to the control throughout the first summer and this effect was attributed to warmer soils after biochar  
398 application to the soil surface (Palviainen et al. 2017a). In the second summer, biochar largely  
399 disappeared under the moss layer (Fig. 1d), and soil temperatures were similar among treatments  
400 (Table 3) which likely reduced the differences in soil CO<sub>2</sub> effluxes between biochar and control plots.  
401 Studies from temperate forest soils have also indicated that increases in soil CO<sub>2</sub> effluxes after biochar  
402 addition are transient and can be generally observed only during the first year (Bruckman et al. 2015;  
403 Page-Dumroese et al. 2017). In the long-term, biochar addition may even decrease the rate of soil C  
404 mineralization because the adsorption of organic matter and microbial extracellular enzymes to  
405 biochar slows down the decomposition (Cross and Sohi 2011; Jones et al. 2011; Zimmerman et al.  
406 2011; Ameloot et al. 2013; Prayogo et al. 2014).

407

408 In growing forests, biochar can only be applied on the soil surface where it may be prone to losses  
409 caused by surface runoff and wind. Bruckman et al. (2016) have studied biochar particle movement  
410 on a forest floor that is very similar to our experiment, by using terrestrial laser scanning in  
411 combination with a time-lapse photography. They used similar biochar as in this study (grain size,  
412 feedstock material, pyrolysis process conditions and post-production procedures) and found that  
413 particle movement is slight and occurs only during heavy precipitation events or strong winds shortly  
414 after biochar application to soil. In this study, little if any biochar was lost from the area with wind  
415 because the forest was quite dense and biochar particles submerged below the ground vegetation and  
416 between the mosses during spreading. The transportation of biochar away with the surface water flow  
417 is also unlikely because the terrain is flat, soil is well-drained coarse sand and there were no heavy  
418 rains during the experimental period. Furthermore, the biochar was not a powder but the particle size

419 was 5-10 mm (Fig. 1a). Bruckman et al. (2015) applied similar biochar as in this study to the soil  
420 surface in a temperate forest, and they found that the litter layer contained more C as compared to the  
421 control plots. This surplus of C equaled the amount which was applied, suggesting that surface applied  
422 biochar effectively incorporates in the organic layer shortly after amendment at the given surface  
423 properties and application rates (Bruckman et al. 2015).

424

425 There was no effect of biochar addition on soil microbial biomass. Previous studies have also found  
426 that biochar additions of 5 to 10 t ha<sup>-1</sup> did not have significant effect on microbial biomass in forest  
427 soils (Sackett et al. 2014; Gundale et al. 2015; Noyce et al. 2015). The null effect on microbial  
428 biomass may be due to the low biochar addition rate. The more pronounced shifts in the soil microbial  
429 biomass have been observed with biochar additions of 20-25 t ha<sup>-1</sup> in temperate forests (Mitchell et  
430 al. 2015, 2016; Page-Dumroese et al. 2017). Many incubation experiments have also indicated  
431 biochar to affect microbial biomass only at high addition rates (Kolb et al. 2009).

432

433 Biochar has often been found to increase soil pH especially in acidic soils (Biederman and Harpole  
434 2013). We found that the addition of 10 t ha<sup>-1</sup> biochar increased soil pH but the biochar amount of 5  
435 t ha<sup>-1</sup> had no effect. Similarly, Noyce et al. (2015) found that the addition of 5 t ha<sup>-1</sup> biochar did not  
436 affect significantly pH in temperate forest soils. Although biochar was applied on the soil surface, it  
437 already had detectable effect on pH in top mineral soil in the second year after treatment in the higher  
438 application rate.

439

440 Although mean N mineralization rates in the organic layer were greater in biochar-amended soils  
441 compared with controls, the data showed large variation and differences between treatments were not  
442 statistically significant ( $P > 0.05$ ). Biochar has been shown to increase soil N immobilization in some  
443 studies (Bruun et al. 2012; Dempster et al. 2012; Zheng et al. 2013; Ameloot et al. 2015), whereas in

444 some studies biochar has increased nitrification and ammonification (Anderson et al. 2011; Nelissen  
445 et al. 2012; Case et al. 2015). Divergent, positive, neutral or negative effects of biochar on N  
446 mineralization in literature may exist due to the C:N-ratio of biochar and the C and N status of the  
447 soil microbes (Prommer et al. 2014). C-rich and N-poor wood biochars may promote N limitation,  
448 leading to the retention of produced ammonium in the N-limited microflora, which therefore results  
449 in a decrease in the amount of ammonium released to the soil. Conversely, N-rich biochars with low  
450 C:N-ratios such as manure-biochars promote microbial C limitation, causing the excess of N to be  
451 mineralized and therefore N mineralization rates to increase (Prommer et al. 2014).

452

453 Several studies have shown that charcoal from wildfires increases nitrification (Berglund et al. 2004;  
454 DeLuca et al. 2002; DeLuca et al. 2006, Ball et al. 2010) likely due to increased soil pH and sorption  
455 of phenolic compounds that inhibit nitrifiers (DeLuca et al. 2006). Contrary to hypothesis, biochar  
456 amendment did not change nitrification rates statistically significantly although soil pH increased.  
457 Possibly the increase in pH was too small to affect the nitrification positively. Liming experiments in  
458 Finland have shown that the pH increase from 4.1 to 4.4 had little effect on N mineralization  
459 (Smolander et al. 1995). Biochar has been even found to decrease nitrification in some studies and it  
460 is suggested that volatile organic compounds (VOC's) contained in biochar or increased ethylene  
461 emissions after biochar addition, inhibit nitrifiers (Clough et al. 2010; Spokas et al. 2010). Biochar  
462 may also limit the nitrifier community by reducing the substrate availability by N adsorption to  
463 biochar surfaces (Laird et al. 2010) and by microbial N immobilization (Kolb et al. 2009). The  
464 observed unchanged nitrification rates suggest that biochar addition does not increase the risk of soil  
465 N losses through nitrate leaching or gaseous losses through denitrification in the studied ecosystem.

466

467 Biological N fixation has been reported to be 0.1–4 kg N ha<sup>-1</sup> yr<sup>-1</sup> in boreal forests (Cleveland et al.  
468 1999; DeLuca et al. 2002, 2008; Zackrisson et al. 2004; Palviainen et al. 2017b). Our results were at

469 the lower end of this range (0.43–0.58 kg N ha<sup>-1</sup> yr<sup>-1</sup>) which may be a consequence of the early  
470 successional stage of the investigated forest stands. The rotation period is 90-100 years, and fire return  
471 interval is 50-200 years in these types of forests (Ohlson et al. 2009). The biological N fixation rates  
472 is estimated to be  $\leq 0.5$  kg ha<sup>-1</sup> yr<sup>-1</sup> in early successional forests (Zackrisson et al. 2004; DeLuca et  
473 al. 2007). Furthermore, the rather high N deposition (7.4 kg ha<sup>-1</sup> yr<sup>-1</sup>) in our study area (Korhonen et  
474 al. 2013) may be one reason for low N fixation rates. N additions of as small as 3 kg N ha<sup>-1</sup> yr<sup>-1</sup> have  
475 already shown to lower N fixation in mosses (Gundale et al. 2011). The addition of biochar did not  
476 have a significant effect on the biomass of mosses although in several studies biochar has been found  
477 to increase the growth of crops and trees (Robertson et al. 2012; Biederman and Harpole 2013;  
478 Thomas and Gale 2015). Our results support the findings of Gundale et al. (2015), who did not find  
479 10 t ha<sup>-1</sup> biochar addition to affect the coverage of mosses in boreal forests. Mosses do not get  
480 advantage for biochar induced improved water holding capacity, cation exchange capacity and  
481 nutrient availability to a similar extent as vascular plants, because boreal mosses are rather drought-  
482 tolerant and receive the majority of their nutrients from rainwater (Brown and Bates 1990).

483

484 To our knowledge, this is the first study to examine the impacts of biochar amendment on biological  
485 N-fixation in boreal forests. Biochar treatments did not have a significant effect on N-fixation which  
486 is likely due to that soil microbial biomass and moss biomass did not markedly change after biochar  
487 addition. In contrast, biochar has been commonly reported to increase N-fixation in leguminous plants  
488 in agro-ecosystems and it has been attributed to elevated soil pH and improved nutrient availability  
489 (Rondon et al. 2007; Mia et al. 2014; Güereña et al. 2015; Van Zwieten et al. 2015). In our study,  
490 biochar increased soil pH which may have had positive effect on N-fixation but on the other hand  
491 part of the biochar contained N may have been mineralized and this may have affected negatively the  
492 N-fixation. Also, Robertson et al. (2012) found that biochar amendment did not change N-fixation  
493 rates in the root nodules of alder seedlings. Nitrogen fixation rates increased with temperature, which

494 is consistent with previous findings that N fixation in feather mosses peaks at temperatures of 13°C–  
495 22°C, and declines above 30°C temperatures (Gentili et al. 2005).

496

497 This study explores short-term responses of biochar amendment in a typical boreal forest. We  
498 conclude that not all potential impacts are evident just one year after biochar application and hence,  
499 specific questions may require long-term experiments. Although our study covered a short response  
500 period relative to a typical forest rotation length, it is an important first step in evaluating the impacts  
501 of potential biochar application in boreal forests on the C and N cycles. Studies like this, in  
502 combination with additional long-term studies, are necessary before biochar use can be promoted and  
503 included in C trading schemes in the boreal region.

504

## 505 **Conclusions**

506

507 The results indicate that wood-derived biochar amendment of 5–10 t ha<sup>-1</sup> did not have a clear and  
508 consistent effect on soil CO<sub>2</sub> effluxes in boreal Scots pine forests. Biochar amendment increased the  
509 soil pH but it had no significant effect on soil microbial biomass and biological N fixation at this  
510 stage. Nitrogen mineralization rates in the organic layer had a tendency to increase with the amount  
511 of added biochar, but no statistically significant effect was detected. The results suggest that biochar  
512 can be utilized to climate change mitigation and C sequestration in boreal forests without causing  
513 undesirable effects on soil microbial biomass, key N cycling processes or native soil C stocks. More  
514 long-term field studies from forest ecosystems are, however, needed to confirm these perceptions and  
515 to find optimum biochar application strategies.

516

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518

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524

## 525 **References**

526

- 527 Adamczyk S, Adamczyk B, Kitunen V, Smolander A (2015) Monoterpenes and higher terpenes  
528 may inhibit enzyme activities in boreal forest soil. *Soil Biol Biochem* 87: 59–66.
- 529 Adamczyk B, Karonen M, Adamczyk S, Engström MT, Laakso T, Saranpää P, Kitunen V,  
530 Smolander A, Simon J (2017) Tannins can slow-down but also speed-up soil  
531 enzymatic activity in boreal forest. *Soil Biol Biochem* 107: 60-67.
- 532 Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR (2011) Biochar  
533 induced soil microbial community change: Implications for biogeochemical cycling of  
534 carbon, nitrogen and phosphorus. *Pedobiologia* 54: 309–320.
- 535 Ameloot N, Graber ER, Verheijen FGA, De Neve S (2013) Interactions between biochar stability  
536 and soil organisms: review and research needs. *Eur J Soil Sci* 64: 379–390.
- 537 Ameloot N, Sleutel S, Das KC, Kanagaratnam J, De Neve S (2015) Biochar amendment to soils  
538 with contrasting organic matter level: effects on N mineralization and biological soil  
539 properties. *GCB Bioenergy* 7: 135–144.
- 540 Ball PN, MacKenzie MD, DeLuca TH, Holben WE (2010) Wildfire and Charcoal Enhance  
541 Nitrification and Ammonium-Oxidizing Bacterial Abundance in Dry Montane Forest  
542 Soils. *J Environ Qual* 39: 1243–1253.
- 543 Beck T, Joergensen RG, Kandeler E, Makeshin E, Nuss E, Oberholzer HR, Scheu S (1997) An

544 inter-laboratory comparison of ten different ways of measuring soil microbial biomass  
545 C. *Soil Biol Biochem* 29: 1023–1032.

546 Berglund LM, DeLuca TH, Zackrisson O (2004) Activated carbon amendments to soil alters  
547 nitrification rates in Scots pine forests. *Soil Biol Biochem* 36: 2067–2073.

548 Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient  
549 cycling: a meta-analysis. *GCB Bioenergy* 5: 202–214.

550 Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release  
551 of soil nitrogen: A rapid direct extraction method to measure microbial biomass  
552 nitrogen in soil. *Soil Biol Biochem* 17: 837–842.

553 Brown DH, Bates JW (1990) Bryophytes and nutrient cycling. *Bot J Linn Soc* 104: 129–147.

554 Bruckman VJ, Terada T, Uzun BB, Apaydin-Varol E, Liu J (2015) Biochar for climate change  
555 mitigation: tracing the in situ priming effect on a forest site. *Energy Procedia* 76: 381–  
556 387.

557 Bruckman VJ, Klinglmüller M, Milenković M (2016) Biochar in the View of Climate Change  
558 Mitigation: the FOREBIOM Experience. In: Bruckman VJ, Apaydin-Varol E, Uzun  
559 BB, Liu J (eds.). *Biochar: A Regional Supply Chain Approach in View of Climate*  
560 *Change Mitigation*. Cambridge University Press: New York, Cambridge. doi:  
561 10.1017/9781316337974.

562 Bruun EW, Ambus P, Egsgaard H, Hauggaard-Nielsen H (2012) Effects of slow and fast pyrolysis  
563 biochar on soil C and N turnover dynamics. *Soil Biol Biochem* 46: 73–79.

564 Cajander AK (1949) Forest types and their significance. *Acta For Fenn* 56.

565 Case SDC, McNamara NC, Reay DS, Stott AW, Grant HK, Whitaker J (2015) Biochar suppresses  
566 N<sub>2</sub>O emissions while maintaining N availability in a sandy loam soil. *Soil Biol*  
567 *Biochem* 81: 178-185.

568 Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty E,



569 Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial  
570 biological nitrogen (N<sub>2</sub>) fixation in natural ecosystems. *Glob Biogeochem Cycles* 13:  
571 623–645.

572 Clough TJ, Condrón LM (2010) Biochar and the Nitrogen Cycle: Introduction. *J Environ Qual*  
573 39: 1218–1223.

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

576 DeLuca TH, Nilsson M-C, Zackrisson O (2002) Nitrogen mineralization and phenol accumulation  
577 along a fire chronosequence in northern Sweden. *Oecologia* 133: 206–214.

578 DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly  
579 influences nitrogen cycling in ponderosa pine forests. *Soil Sci Soc Am J* 70: 448–453.

580 DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson M-C (2007) Ecosystem controls on  
581 nitrogen fixation in boreal feather moss communities. *Oecologia* 152(1): 121–130.

582 DeLuca TH, Zackrisson O, Gundale MJ, Nilsson M-C (2008) Ecosystem Feedbacks and Nitrogen  
583 Fixation in Boreal Forests. *Science* 320: 1181.

584 DeLuca TH, Boisvenue C (2012) Boreal forest soil carbon: distribution, function and modelling.  
585 *Forestry* 85: 161-184.

586 Dempster DN, Gleeson DB, Solaiman ZM, Jones DL, Murphy DV (2012) Decreased soil microbial  
587 biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse  
588 textured soil. *Plant Soil* 354: 311–324.

589 Fang Y, Singha B, Singh BP (2015) Effect of temperature on biochar priming effects and its  
590 stability in soils. *Soil Biol Biochem* 80: 136–145.

591 Gentili F, Nilsson M-C, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular  
592 diversity of feather moss associative N<sub>2</sub>-fixing cyanobacteria. *J Exp Bot* 56(422):  
593 3121–3127.

594 Granhall U Lindberg T (1980) Nitrogen input through biological nitrogen fixation. In: Persson T,  
595 ed. Structure and function of northern coniferous forests – an ecosystem study. Ecol  
596 Bull 32: 333–340.

597 Güereña DT, Lehmann J, Thies JE, Enders A, Karanja N, Neufeldt H (2015) Partitioning the  
598 contributions of biochar properties to enhanced biological nitrogen fixation in  
599 common bean (*Phaseolus vulgaris*). Biol Fertil Soils 51: 479–491.

600 Gundale MJ, DeLuca TH, Nordin A (2011) Bryophytes attenuate anthropogenic nitrogen inputs  
601 in boreal forests. Glob Change Biol 17: 2743–2753.

602 Gundale MJ, Nilsson M-C, Pluchon N, Wardle DA (2015) The effect of biochar management on  
603 soil and plant community properties in a boreal forest. GCB Bioenergy, doi:  
604 10.1111/gcbb.12274.

605 Gurwick NP, Moore LA, Kelly C, Elias P (2013) A Systematic Review of Biochar Research, with  
606 a Focus on Its Stability in situ and Its Promise as a Climate Mitigation Strategy. PLoS  
607 ONE 8(9): e75932. doi:10.1371/journal.pone.0075932.

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

611 He Y, Zhou X, Jiang L, Li M, Du Z, Zhou G, Shao J, Wang X, Xu Z, Bai SH, Wallace H, Xu C  
612 (2017) Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis.  
613 GCB Bioenergy 9: 743–755.

614 Helmisaari H-S, Kaarakka L, Olsson BA (2014) Increased utilization of different tree parts for  
615 energy purposes in the Nordic countries. Scand J For Res 29(4): 312–322.

616 IUSS Working Group WRB (2015) World Reference Base for Soil Resources 2014, update 2015  
617 International soil classification system for naming soils and creating legends for soil  
618 maps. World Soil Resources Reports No. 106. FAO, Rome.

619 Jones DL, Murphy DV, Khalid M, Ahmadd W, Edwards-Jones G, DeLuca TH (2011) Short-  
620 term biochar-induced increase in soil CO<sub>2</sub> release is both biotically and abiotically  
621 mediated. *Soil Biol Biochem* 43: 1723–1731.

622 Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of Charcoal Quantity on Microbial Biomass  
623 and Activity in Temperate Soils. *Soil Sci Soc Am J* 73: 1173–1181.

624 Korhonen JFJ, Pihlatie M, Pumpanen J, Aaltonen H, Hari P, Levula J, Kieloaho A-J, Nikinmaa E,  
625 Vesala T, Ilvesniemi H (2013) Nitrogen balance of a boreal Scots pine forest.  
626 *Biogeosciences* 10: 1083–1095.

627 Kulmala L, Launiainen S, Pumpanen J, Lankreijer H, Lindroth A, Hari P, Vesala T (2008) H<sub>2</sub>O and  
628 CO<sub>2</sub> fluxes at the floor of a boreal pine forest. *Tellus, Series B: Chem Phys Meteorol*  
629 *60 B*: 167–178.

630 Laird D, Fleming P, Wang B, Horton R, Karlen D (2010) Biochar impact on nutrient leaching from  
631 a Midwestern agricultural soil. *Geoderma* 158: 436–442.

632 Lehmann J, Rillig MC, Thies J, Masiello CA, Kockaday WC, Crowley D (2011) Biochar effects on  
633 soil biota – a review. *Soil Biol Biochem* 43: 1812–1836.

634 Lehmann J, Joseph S (2012) *Biochar for Environmental Management – Science and Technology*.  
635 MBG Books, UK. 415 p.

636 Lei O, Zhang R (2013) Effects of biochars derived from different feedstocks and pyrolysis  
637 temperatures on soil physical and hydraulic properties. *J Soils Sediments* 13: 1561–  
638 1572.

639 Leppänen SM, Salemaa M, Smolander A, Mäkipää R, Tirola M (2013) Nitrogen fixation and  
640 methanotrophy in forest mosses along a N deposition gradient. *Environ Exp Bot* 90:  
641 62–69.

642 Limmer C, Drake HL (1996) Nonsymbiotic N<sub>2</sub>-fixation in acidic and pH-neutral forest soils:  
643 aerobic and anaerobic differentials. *Soil Biol Biochem* 28: 177–183.

644 Liu S, Zhang Y, Zong Y, Hu Z, Wu S, Zhou JIE, Jin Y, Zou J (2015) Response of soil carbon  
645 dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar  
646 amendment: a meta-analysis. *GCB Bioenergy*, doi: 10.1111/gcbb.12265

647 Mia S, van Groenigen JW, van de Voorde TFJ, Oram NJ, Bezemer TM, Mommer L, Jeffery  
648 S (2014) Biochar application rate affects biological nitrogen fixation in red clover  
649 conditional on potassium availability. *Agriculture, Ecosyst Environ* 191: 83–91.

650 Mitchell PJ, Simpson AJ, Soong R, Simpson MJ (2015) Shifts in microbial community and water-  
651 extractable organic matter composition with biochar amendment in a temperate forest  
652 soil. *Soil Biol Biochem* 81: 244-254.

653 Mitchell PJ, Simpson AJ, Soong R, Schurman JS, Thomas SC, Simpson MJ (2016) Biochar  
654 amendment and phosphorus fertilization altered forest soil microbial community and  
655 native soil organic matter molecular composition. *Biogeochem* 130: 227–245.

656 Nelissen V, Rütting T, Huygens D, Staelens J, Ruyschaert G, Boeckx P. (2012) Maize biochars  
657 accelerate short-term soil nitrogen dynamics in a loamy sand soil. *Soil Biol Biochem*  
658 55: 20–27.

659 Nohrstedt H-Ö (1985) Nonsymbiotic nitrogen fixation in the topsoil of some forest stands in central  
660 Sweden. *Can J For Res* 15: 715–722.

661 Noyce GL, Basiliko N, Fulthorpe R, Sackett TE, Thomas SC (2015) Soil microbial responses over  
662 2 years following biochar addition to a north temperate forest. *Biol Fertil Soils* 51:  
663 649–659.

664 Ohlson M, Dahlberg B, Økland T, Brown KJ, Halvorsen R (2009) The charcoal carbon pool in  
665 boreal forest soils. *Nature Geoscience* 2: 692-695.

666 Page-Dumroese DS, Coleman MD, Thomas SC (2017) Opportunities and uses of biochar on forest

667 sites in North America [Chapter 15]. In: Bruckman V, Varol EA, Uzun B, Liu J (eds.)  
668 Biochar: A Regional Supply Chain Approach in View of Climate Change Mitigation.  
669 Cambridge, UK: Cambridge University Press. p. 315-335.

670 Palviainen M, Berninger F, Zhu T, Köster K, Bruckman V, Moreira De Assumpção C, Mishra A,  
671 Pumpanen J (2017a) Short-term effects of biochar on carbon and nitrogen cycling in  
672 boreal Scots pine forests. BIOGEOMON 2017, 9th International Symposium on  
673 Ecosystem Behavior, August 20-24, 2017, Litomyšl, Czech Republic. Book of  
674 abstracts pp. 182-183. <http://www.biogeomon.cz/documents/Abstracts.pdf>

675 Palviainen M, Pumpanen J, Berninger F, Ritala K, Duan B, Heinonsalo J, Sun H, Köster E,  
676 Köster K (2017b) Nitrogen balance along a northern boreal forest fire  
677 chronosequence. PLOS ONE <https://doi.org/10.1371/journal.pone.0174720>.

678 Pirinen P, Simola H, Aalto J, Kaukoranta J-P, Karlsson P, Ruuhela R (2012) Climatological  
679 statistics of Finland 1981-2010. Finnish Meteorological Institute, Helsinki, Finland.

680 Prayogo C, Jones JE, Baeyens J, Bending GD (2014) Impact of biochar on mineralisation of C  
681 and N from soil and willow litter and its relationship with microbial community  
682 biomass and structure. *Biol Fertil Soils* 50: 695–702.

683 Priha O, Smolander A (1995) Nitrification, denitrification and microbial biomass N in soil from  
684 two N-fertilized and limed Norway spruce forests. *Soil Biol Biochem* 27: 305–310.

685 Prommer J, Wanek W, Hofhansl F, Trojan D, Offre P, Urich T, Schleper C, Sassmann S, Kitzler B,  
686 Soja G, Hood-Nowotny RC (2014) Biochar Decelerates Soil Organic Nitrogen  
687 Cycling but Stimulates Soil Nitrification in a Temperate Arable Field Trial. *PLoS*  
688 *ONE* 9(1): e86388. doi:10.1371/journal.pone.0086388.

689 Pumpanen J, Kulmala L, Lindén A, Kolari P, Nikinmaa E, Hari P (2015) Seasonal dynamics of  
690 autotrophic respiration in boreal forest soil estimated by continuous chamber  
691 measurements. *Bor Env Res* 20: 637–650.

692 Robertson SJ, Rutherford PM, López-Gutiérrez JC, Massicotte HB (2012) Biochar enhances  
693 seedling growth and alters root symbioses and properties of sub-boreal forest soils.  
694 Can J Soil Sci 92: 329–340.

695 Rondon MA, Lehmann J, Ramirez J, Hurtado M (2007) Biological nitrogen fixation by common  
696 beans (*Phaseolus vulgaris* L) increases with bio-char additions. Biol Fertil Soils 43:  
697 699–708.

698 Sackett TE, Basiliko N, Noyce GL, Winsborough C, Ikeda C, Thomas SC (2014) Soil and  
699 greenhouse gas responses to biochar additions in a temperate hardwood forest. GCB  
700 Bioenergy, doi: 10.1111/gcbb.12211.

701 Smith JL, Collins HP, Bailey VL (2010) The effect of young biochar on soil respiration. Soil  
702 Biol Biochem 42: 2345–2347.

703 Smolander A, Kitunen V, Priha O, Mälkönen E (1995) Nitrogen transformations in limed and  
704 nitrogen fertilized soil in Norway spruce stands. Plant Soil 17: 107–115.

705 Song X, Pan G, Zhang C, Zhang L, Wang H (2016) Effects of biochar application on fluxes of three  
706 biogenic greenhouse gases: a meta-analysis. Ecosyst Health Sustainability  
707 2(2):e01202. doi: 10.1002/ehs2.1202

708 Spokas KA, Reicosky DC (2009) Impacts of sixteen different biochars on soil greenhouse gas  
709 production. Ann Env Sci 3: 179–193.

710 Spokas KA, Baker JM, Reicosky DC (2010) Ethylene: potential key for biochar amendment  
711 impacts. Plant Soil 333: 443–452.

712 Sponseller RA, Gundale MJ, Futter M, Ring E, Nordin A, Näsholm T, Laudon H (2016)  
713 Nitrogen dynamics in managed boreal forests: Recent advances and future research  
714 directions. Ambio 45(Suppl. 2): S175–S187.

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

717 Stewart CE, Zheng JY, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar miti-  
718 gates green-house gas emissions and increases carbon sequestration in temperate soils.  
719 Glob Change Biol Bioenergy 5: 153–164.

720 Thomas SC, Gale G (2015) Biochar and forest restoration: a review and meta-analysis of tree  
721 growth responses. New Forests 46: 931–946.

722 Zackrisson O, Nilsson MC, Wardle DA (1996) Key ecological function of charcoal from wildfire in  
723 the boreal forest. Oikos 77:10–19.

724 Zackrisson O, DeLuca TH, Nilsson M-C, Sellstedt A, Berglund LM (2004) Nitrogen fixation  
725 increases with successional age in boreal forests. Ecology 85: 3327–3334.

726 Zheng H, Wang Z, Deng X, Herbert S, Xing BS (2013) Impacts of adding biochar on nitrogen  
727 retention and bioavailability in agricultural soil. Geoderma 206: 32–39.

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

730 Van Zwieten L, Rose T, Herridge D, Kimber S, Rust J, Cowie A, Morris S (2015) Enhanced  
731 biological N<sub>2</sub> fixation and yield of faba bean (*Vicia faba* L.) in an acid soil following  
732 biochar addition: dissection of causal mechanisms. Plant Soil 395: 7–20.

733 Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil  
734 microbial biomass C. Soil Biol Biochem 19(6): 703–707.

735 Wang J, Xiong Z, Kuzyakov (2015) Biochar stability in soil: meta-analysis of decomposition and  
736 priming effects. GCB Bioenergy, doi: 10.1111/gcbb.12266

737 Wardle DA, Nilsson M, Zackrisson O (2008) Forest-derived charcoal causes loss of forest humus.  
738 Science 320: 629.

739 Woolf D, Amonette JE, Stree-Perrott FA, Lehmann J, Joseph S (2010) Sustainable biochar to  
740 mitigate global climate change. Nat. Comm. 1: 56.

741

742 Table 1. Mean ( $\pm$ SE) soil carbon and nitrogen concentrations, carbon and nitrogen ratio, soil carbon  
 743 and nitrogen pools and soil particle size distribution in the study site.

744

Soil layer	C (%)	N (%)	C:N ratio	C g m <sup>2</sup>	N g m <sup>2</sup>	Clay (%)	Silt (%)	Sand (%)
Organic layer	29.82 (1.45)	0.91 (0.04)	33 (0.5)	672 (53)	20 (1)			
Mineral soil 0– 5 cm	3.23 (0.32)	0.11 (0.01)	29 (0.8)	737 (72)	25 (3)	0.00	15.48	84.52
Mineral soil 5– 15 cm	1.28 (0.06)	0.05 (0.003)	26 (8.7)	746 (39)	27 (2)	0.00	12.67	87.33

745

746



747 Table 2. Characteristics of the biochars. Values are mean  $\pm$  SE.

748

	Biochar 500°C	Biochar 650°C
C (%)	60.61 (1.93)	60.61 (1.88)
N (%)	0.87 (0.03)	0.29 (0.10)
C:N ratio	70 (4.1)	364 (70.4)
Loss on ignition (LOI)	82.0 (6.4)	87.5 (1.4)
pH	8.77 (0.23)	8.87 (0.07)
Electric conductivity $\mu\text{s cm}^{-1}$	1361 (226)	1462 (179)
Al $\text{mg g}^{-1}$	21.83 (1.68)	20.78 (1.07)
Ca $\text{mg g}^{-1}$	190.68 (10.34)	154.15 (7.17)
Cu $\text{mg g}^{-1}$	0.22 (0.01)	0.20 (0.01)
Fe $\text{mg g}^{-1}$	24.01 (2.09)	21.32 (1.10)
K $\text{mg g}^{-1}$	29.81 (3.27)	42.87 (6.73)
Mg $\text{mg g}^{-1}$	17.08 (1.09)	19.32 (1.55)
Mn $\text{mg g}^{-1}$	3.86 (0.16)	2.95 (0.10)
Na $\text{mg g}^{-1}$	3.30 (0.96)	2.71 (0.24)
Ni $\text{mg g}^{-1}$	0.07 (0.01)	0.08 (0.01)
P $\text{mg g}^{-1}$	10.78 (0.40)	7.41 (0.21)
S $\text{mg g}^{-1}$	5.61 (0.25)	4.33 (0.12)
Si $\text{mg g}^{-1}$	2.29 (0.11)	2.19 (0.05)
Zn $\text{mg g}^{-1}$	0.42 (0.01)	0.34 (0.01)

749

750

751 Table 3. Monthly mean ( $\pm$ SE) soil temperatures ( $^{\circ}$ C) at 5 cm depth in different treatments. The same  
752 letters indicate no statistically significant differences among treatments.

753

Treatment	May	June	July	August	September
Control	7.0 (0.4) <sup>a</sup>	11.7 (0.4) <sup>a</sup>	14.7 (0.2) <sup>a</sup>	13.8 (0.2) <sup>a</sup>	10.2 (0.3) <sup>a</sup>
Biochar 500 $^{\circ}$ C 5 t ha <sup>-1</sup>	8.4 (0.4) <sup>a</sup>	12.3 (0.4) <sup>a</sup>	15.2 (0.2) <sup>a</sup>	14.3 (0.2) <sup>a</sup>	10.5 (0.3) <sup>a</sup>
Biochar 500 $^{\circ}$ C 10 t ha <sup>-1</sup>	8.1 (0.4) <sup>a</sup>	12.8 (0.4) <sup>a</sup>	15.6 (0.2) <sup>a</sup>	14.4 (0.2) <sup>a</sup>	10.4 (0.3) <sup>a</sup>
Biochar 650 $^{\circ}$ C 5 t ha <sup>-1</sup>	7.3 (0.5) <sup>a</sup>	12.5 (0.4) <sup>a</sup>	15.3 (0.2) <sup>a</sup>	14.4 (0.2) <sup>a</sup>	10.6 (0.3) <sup>a</sup>
Biochar 650 $^{\circ}$ C 10 t ha <sup>-1</sup>	7.5 (0.4) <sup>a</sup>	11.5 (0.4) <sup>a</sup>	14.5 (0.2) <sup>a</sup>	13.9 (0.2) <sup>a</sup>	10.5 (0.3) <sup>a</sup>

754

755

756 Table 4. The average biomass ( $\pm$  SE) of mosses ( $\text{kg ha}^{-1}$ ) in different treatments.

757

	Control	Biochar 5 t $\text{ha}^{-1}$	Biochar 10 t $\text{ha}^{-1}$
<i>Pleurozium schreberi</i>	2588 (497) <sup>ab</sup>	4313 (588) <sup>a</sup>	2451 (593) <sup>b</sup>
<i>Dicranum polysetum</i>	1978 (801) <sup>a</sup>	1000 (557) <sup>a</sup>	1524 (587) <sup>a</sup>
Total biomass of mosses	4566 (455) <sup>a</sup>	5313 (572) <sup>a</sup>	3975 (582) <sup>a</sup>

758

759

760 Figure captions

761 **Figure 1. a)** The grain size of the biochar was 5-10 mm **b)** Biochar was spread manually on the soil  
762 surface **c)** Study site **d)** The mosses grew on top of the biochar layer one year after biochar addition.

763 **Figure 2.** Mean ( $\pm$ SE) soil CO<sub>2</sub> effluxes in different treatments in June and July. Different letters  
764 indicate statistically significant differences ( $P < 0.05$ ) between treatments.

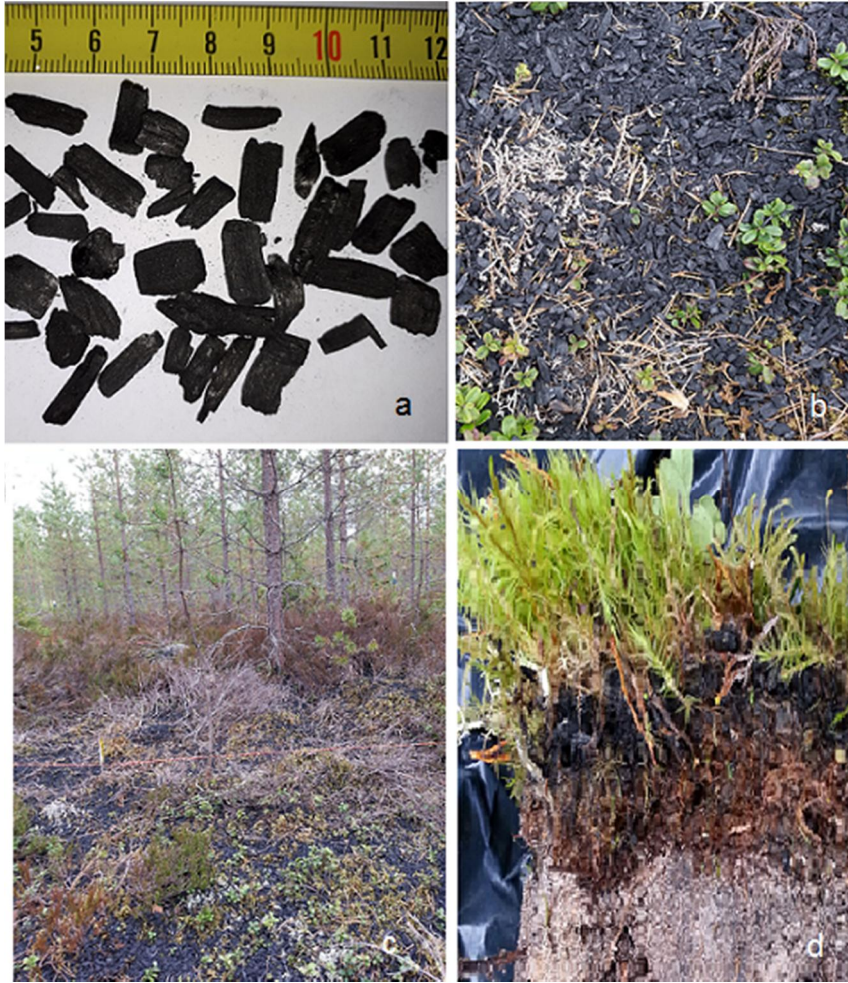
765 **Figure 3.** Mean ( $\pm$ SE) soil microbial biomass carbon, soil microbial biomass nitrogen and soil  
766 microbial biomass carbon:nitrogen ratio in control plots and 5 t ha<sup>-1</sup> and 10 t ha<sup>-1</sup> biochar (pyrolysis  
767 temperature 650°C) treatments. Different letters indicate statistically significant differences ( $P < 0.05$ )  
768 between treatments.

769 **Figure 4.** The mean ( $\pm$ SE) biological nitrogen fixation rates ( $\mu\text{g m}^{-2} \text{d}^{-1}$ ) in each incubation  
770 temperature (10°C, 15°C and 20°C) in control plots and 5 t ha<sup>-1</sup> and 10 t ha<sup>-1</sup> biochar (pyrolysis  
771 temperature 650°C) treatments. Statistically significant differences ( $P < 0.05$ ) between treatments in  
772 each temperature group are indicated by different lower-case letters, whereas statistically significant  
773 differences ( $P < 0.05$ ) between temperatures are indicated by upper-case letters in parenthesis.

774 **Figure 5.** The mean ( $\pm$ SE) soil pH in the organic layer and upper 10 cm mineral soil layer in control  
775 plots and 5 t ha<sup>-1</sup> and 10 t ha<sup>-1</sup> biochar (pyrolysis temperature 650°C) treatments. Different letters  
776 indicate statistically significant differences ( $P < 0.05$ ) between treatments.

777 **Figure 6.** The mean ( $\pm$ SE) net nitrogen mineralization, ammonification and nitrification rates in the  
778 organic layer and upper 10 cm mineral soil layer in control plots and 5 t ha<sup>-1</sup> and 10 t ha<sup>-1</sup> biochar  
779 (pyrolysis temperature 650°C) treatments. Different letters indicate statistically significant  
780 differences ( $P < 0.05$ ) between treatments.

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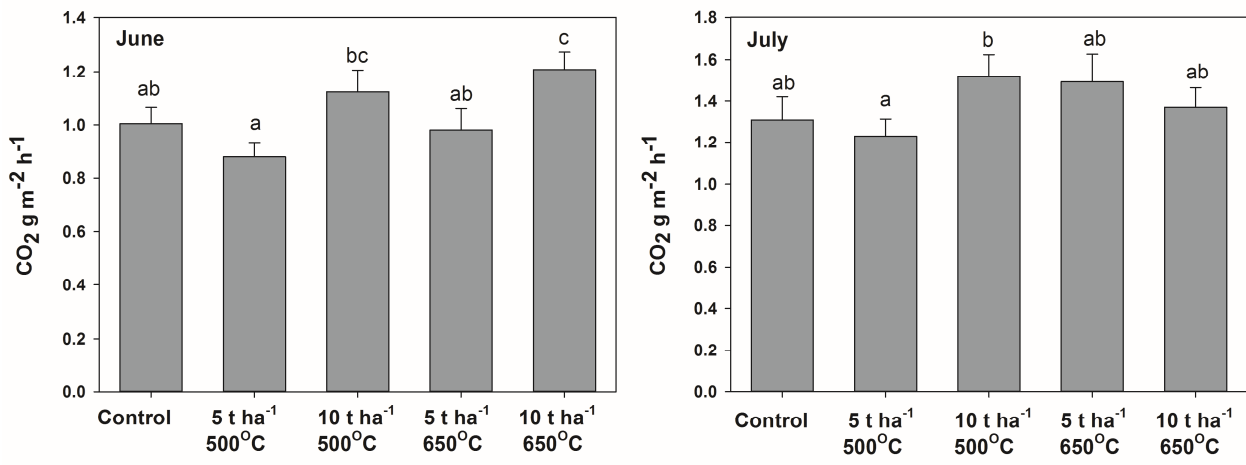
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Fig. 1

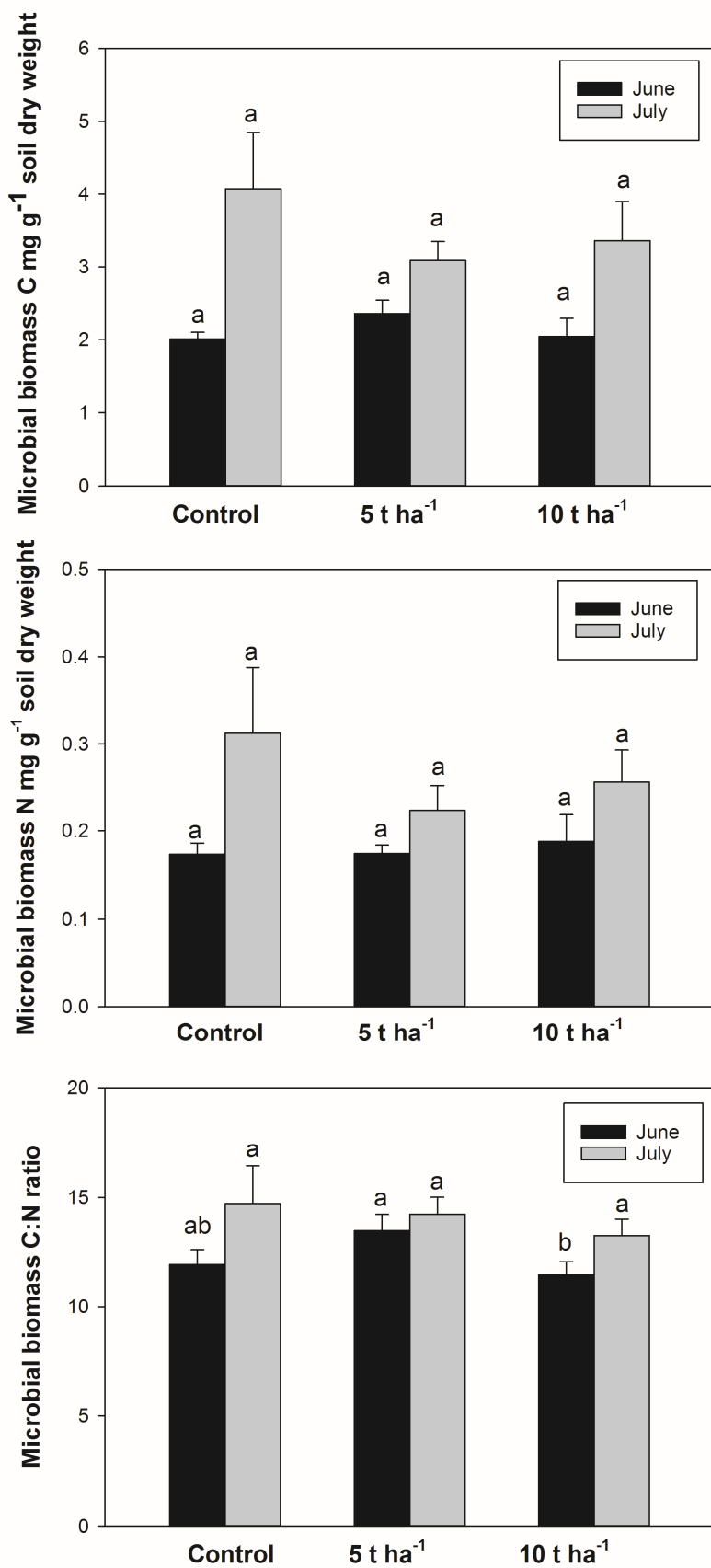
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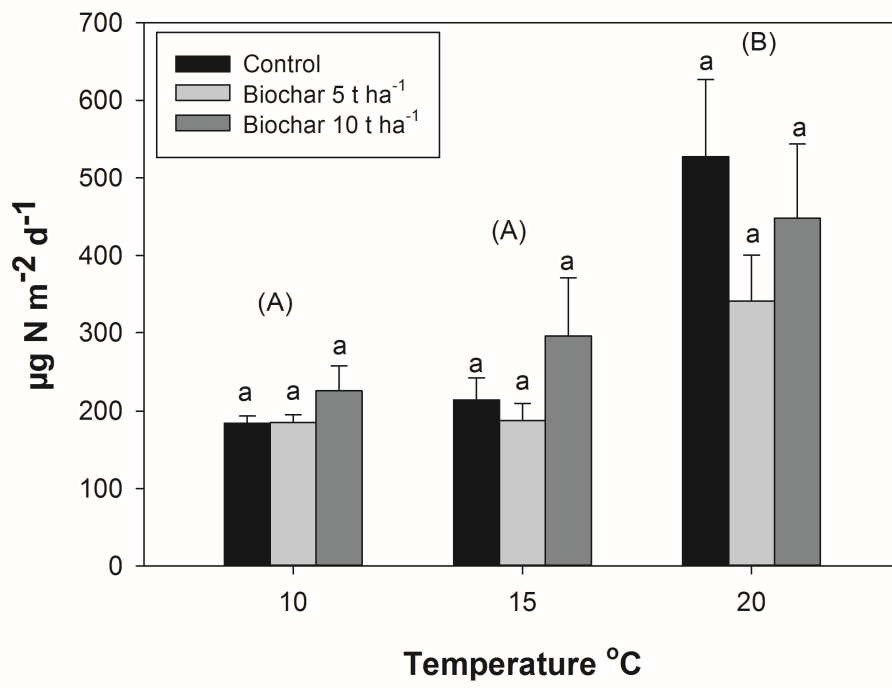
788 Fig. 2

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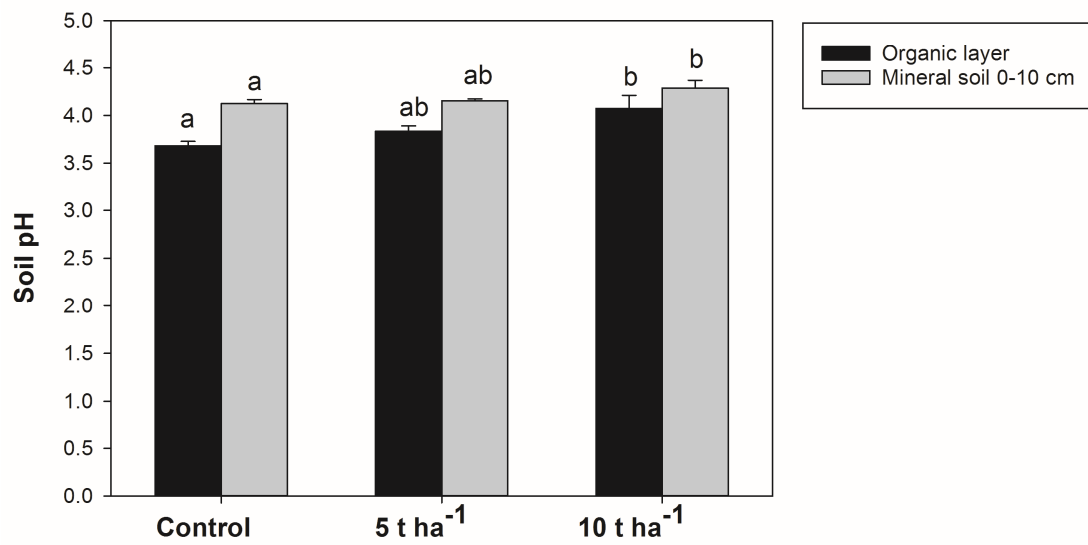
791 Fig.3



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793 Fig. 4

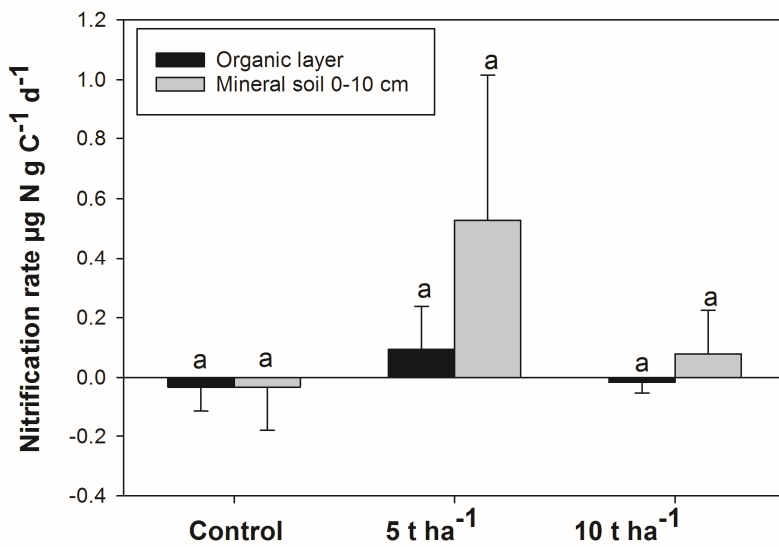
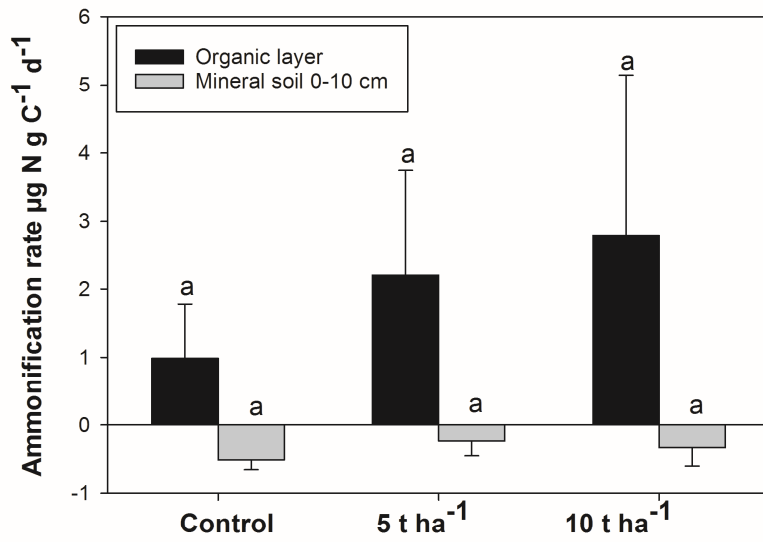
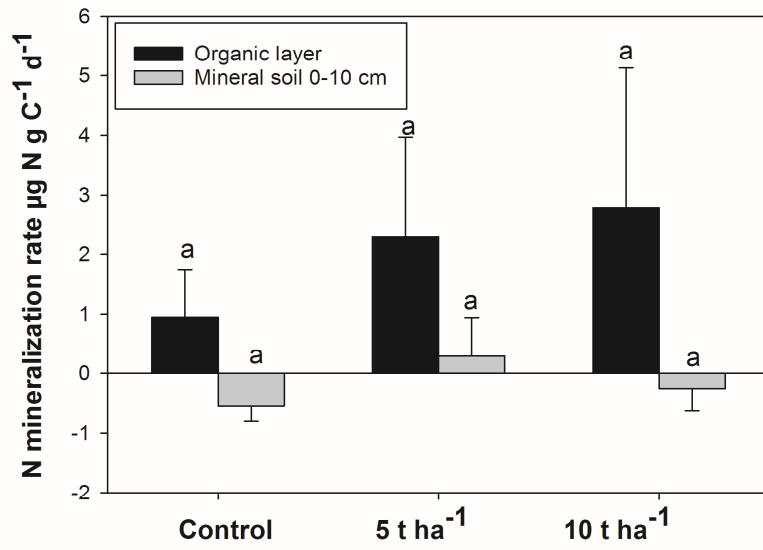




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795 Fig. 5

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798 Fig. 6