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

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Abstract

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

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

Results During the second summer after treatment, soil CO_2 effluxes showed no clear response to biochar addition. Only in June, the 10 t ha^{-1} biochar (650°C) plots had significantly higher CO_2 effluxes compared to the control plots. The pyrolysis temperature of biochar did not affect soil CO_2 effluxes. Soil pH increased in the plots receiving 10 t ha^{-1} biochar additions. Biochar treatments had no significant effect on soil microbial biomass and biological N fixation. Nitrogen mineralization rates in the organic layer tended to increase with the amount of biochar, but no statistically significant effect was detected.

Conclusions The results suggest that wood biochar amendment rates of 5–10 t ha^{-1} to boreal forest soil do not cause large or long-term changes in soil CO_2 effluxes or reduction in native soil C stocks. Furthermore, the results imply that biochar does not adversely affect soil microbial biomass or key N cycling processes in boreal xeric forests, at least within this time frame. Thus, it seems that biochar is a promising tool to mitigate climate change and sequester additional C in boreal forest soils.

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

Introduction

Biochar is formed by heating organic material under low oxygen concentrations in a process known as pyrolysis (Lehmann and Joseph 2012). The addition of biochar to soil is a potential tool for carbon (C) sequestration and climate change mitigation because biochar is enriched in C and recalcitrant to decomposition in comparison to the original biomass (Woolf et al. 2010; Gurwick et al. 2013). Biochar can also act as a soil conditioner enhancing plant growth by increasing soil microbial activity, water holding capacity, cation exchange capacity and pH (Lehmann and Joseph 2012; Robertson et al. 2012; Biederman and Harpole 2013; Thomas and Gale 2015). However, these changes in soil chemical and physical properties may increase microbial biomass, microbial activity and the decomposition of soil organic matter (Lehmann and Joseph 2012). Moreover, the labile C fractions of biochar may accelerate the decomposition of old soil organic matter through the priming effect (Cross and Sohi 2011; Zimmerman et al. 2011; Fang et al. 2015; Wang et al. 2015). In addition, biochar may affect the chemistry of phenolic compounds which commonly inhibit the decomposition of soil organic matter in boreal forest soils. Fire-derived charcoal have been found to adsorb phenolic compounds and to accelerate organic matter decomposition in boreal forests (Zackrisson et al. 1996; Wardle et al. 1998, 2008). Accelerated decomposition of native soil C increases soil CO_2 emissions and reduces the soil C stocks, which is contradicting the idea of C sequestration.

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

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

In boreal forests, most of the soil nitrogen (N) is in organic form, N mineralization rates are low and tree growth is N-limited (Sponseller et al. 2016). The mineralization of N can be accelerated if biochar stimulates soil organic matter decomposition which, in turn, may have a positive feedback on ecosystem net primary production and CO_2 fixation. Biochar application has been shown to increase net N mineralization and nitrification rates (Ameloot et al. 2015; Case et al. 2015; Gundale et al. 2015) which has been attributed to increased soil pH, enhanced microbial growth and activity and the sorption of phenols and terpenes onto biochar (Clough and Condron 2010; Lehmann et al. 2011). Polyphenolics and terpenes inhibit nitrification and net N mineralization by decreasing the activity of enzymes involved in N cycling (Adamczyk et al. 2015, 2017). Wildfire-produced charcoal has been found to adsorb phenols, and to increase net N mineralization and nitrification in forest soils (Zackrisson et al. 1996; Wardle et al. 1998; DeLuca et al. 2006; Ball et al. 2010). Biochar may thus serve as an important soil amendment, and it could be possibly used for mimicking the effects of fire-derived charcoal in Finland, where forest fires are effectively controlled (total area of forest fires is only 300-1000 ha^{-1} yr^{-1}) and forest soils contain high amounts of phenolic compounds. On the other hand, the reduction of N mineralization and increased N immobilization may occur when biochar compounds with a high C:N ratio are microbially degraded (Bruun et al. 2012; Dempster et al. 2012; Prommer et al. 2014) and due to the adsorption of NH_4^+ or NO_3^- onto the biochar surface (Clough and Condron 2010).

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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_2 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_2 effluxes for a longer term in boreal forest soil.

Materials and methods

Study area

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

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

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

Soil and biochar analyses

Soil samples were collected from the organic layer and the upper 15 cm mineral soil layer using stainless soil corer (diameter 5.5 cm) at nine locations in each subplot in mid-May in 2015 just before biochar addition. The samples were dried (60°C, 24 h), sieved through a 2-mm sieve, and ground before the analysis. Subsamples were taken for dry mass determination at 105ºC. Soil particle size distribution was determined by the laser diffraction (LS230, Coulter Corp., Miami, Florida, USA) method (Table 1). The C and N concentrations of soil and biochars were analyzed with an elemental analyzer (Vario Max CN elemental analyser, Elementar Analysensysteme GmbH, Germany). The loss on ignition (LOI) of biochars were determined by combusting samples at 550°C for 3 hours. The concentrations of P, K, Ca, Mg, S, Fe, Al, Na, Cu, Mn, Ni, Si and Zn in biochar were determined from HNO_3-H_2O_2 digestion by ICP atomic emission spectrophotometer (ARL 3580 OES, Fison Instruments, Valencia, USA). Biochar pH was determined using a pH meter (PHM210, Radiometer Analytical, France) on a 1:2.5 (v:v) biochar /water solution and electric conductivity was measured

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

Soil temperature and soil respiration measurements

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

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

Soil respiration i.e. CO_2 effluxes were measured with a closed chamber system consisting of an opaque cylindrical polycarbonate chamber (diameter 20 cm, height 30 cm), a CO_2 analyzer, sensor for relative humidity and temperature and a data logger (Kulmala et al. 2008; Pumpanen et al. 2015). The CO_2 concentration inside the chamber was recorded with a GMP343 diffusion type CO_2 probe (Vaisala Oy, Vantaa, Finland) at 5-second intervals and corrected automatically for humidity, temperature and pressure with a data recorder (MI70, Vaisala Oyj) using the readings from the 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 ± 0.7°C in June and ± 1.0°C in July, and the variation in soil temperatures was even smaller
204 (±0.3°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 CO_2 efflux was calculated as the slope of a linear regression of
208 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
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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°C
215 produced biochar were added 5 t ha^{-1} and 10 t ha^{-1}, respectively. Soil samples were stored at +5°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/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°C)

and moisture (soil moisture content adjusted to 60% of the water-holding capacity) for 42 days. At the start and at the end of the incubation, an analysis of inorganic N was performed to estimate net N mineralization, ammonification and nitrification for the samples. Each soil sample was extracted with 40 ml of 1 M KCl for 2 h (ISO 14256–2: 2005). The KCl extracts were filtered through a 0.45-µm filter and ammonium (NH_4-N) and nitrate (NO_3-N) concentrations were analyzed with a flow-injection ion analyzer (Lachat Quickchem 8000, Milwaukee, WI, USA). Initial concentrations of (NH_4^+-N) and (NO_3^--N) were subtracted from the corresponding post-incubation concentrations to calculate the rates of net ammonification and nitrification. Net mineralized N was calculated from the sum of (NH_4^+-N) and (NO_3^--N) accumulated during the period of incubation. The incubated soil samples were dried, ground with a mortar grinder (Retsch RMO Mortar Grinder, Retsch GmbH, Germany) and their C and N concentrations were measured with an elemental analyser (Vario Max CN, Elementar Analysensysteme GmbH, Germany). A subsample was taken for dry mass determination (105°C, 24 h). The formed inorganic N was expressed on organic matter basis (µg N, NO_3 or NH_4 g C^{-1} d^{-1}).

Soil pH was measured from separate samples by mixing 10 ml of soil with 25 ml of deionized water. The suspension pH (H_2O) was measured with a glass electrode (PHM210, Radiometer Analytical, France) after 24 hours.

Biological nitrogen fixation and moss biomass

The samples containing mosses and organic layer were collected in May, June and July 2016 with a soil core cylinder (diameter 5.8 cm) from the control subplots and from the subplots where 650°C produced biochar were added 5 t ha^{-1} and 10 t ha^{-1}, respectively. In total, 108 samples were collected 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.
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263 Soil microbial biomass
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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).

Soil microbial biomass C and N were determined by a chloroform fumigation extraction method (Brookes et al. 1985; Vance et al. 1987). Three grams of soil from each sample was weighed, placed into glass beakers and fumigated with 30 ml ethanol-free chloroform ($CHCl_3$) in a vacuum desiccator. Another equivalent sample weighting three grams was placed in plastic bottles in another desiccator as un-fumigated control samples. Both desiccators were kept at 25 °C in the dark for 24 hours. After fumigation, 0.5 M potassium sulfate (K_2SO_4) (with the ratio of oven-dry basis soil: K_2SO_4=1:20) was used to extract the fumigated and un-fumigated samples. Then the samples were shaken at 200 rpm for 1 hour and filtered using Whatman No.42 ashless filter papers. The filtrate was then used to analyze the microbial C and N by a TOC-VCPH analyzer (Shimadzu Corp., Kyoto, Japan). Microbial biomass C and N were calculated as the difference between fumigated and unfumigated samples and the difference was divided by the soil-specific calibration factor which was 0.45 for C (Beck et al. 1997) and 0.54 for N (Brookes et al. 1985).

Statistical analyses

The effect of biochar amendment on soil pH, soil temperature, soil respiration, soil microbial biomass, biological N fixation and N mineralization were analyzed with linear mixed model followed by Fisher's least significant difference (LSD) test. Treatment was a fixed factor and plot was a random factor. In the soil respiration analyses, the collar within the subplot was set as random factor. Data were checked for normality with the Shapiro–Wilk test and the recorded CO_2 effluxes were logarithm-transformed. Differences were considered statistically significant when P was ≤ 0.05. Statistical tests were performed using IBM SPSS version 23 (IBM Corp, Armonk, NY, USA). The results of the statistical tests are presented in supplementary material.

Results

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297 Biochar characteristics

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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^{-1} and 6061 kg ha^{-1} of C
302 were added to the soil along with 5 t and 10 t ha^{-1} 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).

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306 Soil temperature and soil respiration

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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_2 efflux was
312 significantly higher in plots where 650°C produced biochar was applied 10 t ha^{-1} compared to control
313 and 5 t ha^{-1} biochar treatments (Fig. 2). Both in June and July, 500°C biochar plots had higher soil
314 CO_2 effluxes in 10 t ha^{-1} treatments compared to 5 t ha^{-1} treatments (Fig. 2). The production
315 temperature of biochar did not have an effect on soil CO_2 fluxes, as there was no statistically
316 significant difference in CO_2 effluxes between 500°C and 650°C biochar subplots in 5 t and 10 t ha^{-1}
317 treatments.

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319 Soil microbial biomass, moss biomass and biological N fixation

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The biochar treatments did not significantly influence soil microbial biomass C or soil microbial biomass N (Fig. 3). Microbial biomass C:N-ratio was significantly higher in 5 t ha^{-1} biochar plots than in 10 t ha^{-1} biochar plots in June, but microbial biomass C:N-ratios did not differed between treatments in July (Fig. 3).

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

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

Soil pH and N transformations

Soil pH in the organic layer and the upper 10 cm mineral soil layer was significantly higher ($P < 0.04$) in 10 t ha^{-1} treated biochar plots than in the control plots (Fig. 5). In the control plots, soil pH was 3.7 in the organic layer and 4.1 in the upper 10 cm mineral soil, whereas in 10 t ha^{-1} treated biochar plots the respective values were 4.1 and 4.3.

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

Discussion

Few studies have investigated *in-situ* the effects of biochar addition on soil respiration in forest ecosystems. There was no clear and consistent tendency towards increased soil CO_2 effluxes during the second summer after biochar addition. Only in June, the CO_2 effluxes were significantly (17%) higher in 10 t ha^{-1} 650°C produced biochar plots than in the control plots. Otherwise, there were no differences in soil CO_2 effluxes between control and biochar treatments. Slightly increased soil CO_2 effluxes after biochar addition may be observed due to the mineralization of labile C fractions of biochar and/or biochar induced priming effects in the soil shortly after biochar amendment (Smith et al. 2010; Zimmerman 2011; Cross and Sohi 2011; Jones et al. 2011). Biochar may also indirectly stimulate microbial activity by providing nutrients, offering a habitat because of its porous structure, increasing soil pH and reducing the bioavailability of toxic compounds in soil through sorption (Steinbeiss et al. 2009; Lehmann et al. 2011; Lehmann and Joseph 2012; Hammer et al. 2014). In addition, biochar may increase plant growth and root biomass (Lehmann et al. 2011; Robertson et al. 2012; Thomas and Gale 2015), which promotes root respiration and provides additional organic matter for decomposition.

Both increased and decreased C mineralization has been observed following biochar addition to various types of soils (Cross and Sohi 2011; Zimmerman et al. 2011; Liu et al. 2015; Wang et al. 2015). Studies from temperate forests have reported short-term positive priming effects or unchanged soil respiration after biochar addition (Sackett et al. 2014; Bruckman et al. 2015). Gundale et al. (2015) mixed 10 t ha^{-1} biochar to boreal forest soil and did not find significant effect on soil respiration. In general, the positive priming effects are observed in soils which have low C contents (Zimmerman et al. 2011). Weak priming effects and moderate changes in CO_2 effluxes in boreal forest soils after biochar addition may take place since boreal forest soils have high C content (DeLuca and Boisvenue 2012).

The responses of soil CO_2 effluxes depend also on feedstock characteristics, pyrolysis temperature and application rate (Zimmerman et al. 2011; He et al. 2017). In general, wood biochars increase soil CO_2 effluxes to a lesser degree compared to other types of biochars, and soil CO_2 effluxes decline with biochar pyrolysis temperature (Zimmerman et al. 2011; He et al. 2017). In the present study, wood biochar, produced at relatively high temperatures, may be the reason for the small changes in soil respiration. Furthermore, apparently moderate biochar amendments do not cause large increases in soil respiration. For example, meta-analyses from croplands have showed that biochar increases soil CO_2 emissions significantly only at high (20–40 t ha^{-1}) amendment rates (Song et al. 2016; He et al. 2017). However, our results also showed that there was tendency for higher soil CO_2 effluxes from 10 t ha^{-1} plots than from 5 t ha^{-1} plots, at least in 500°C biochar treatments (Fig. 2). Pyrolysis temperature did not have an effect on soil CO_2 fluxes, although generally biochars produced at high (> 600°C) temperatures are more recalcitrant than those produced at lower temperatures (Cross and Sohi 2011; Ameloot et al. 2013; Fang et al. 2015) and they often cause negative priming effects in the soil (Zimmerman et al. 2011; Song et al. 2016).

394 The differences in soil CO_2 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_2 effluxes at 10 t ha^{-1}
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_2 effluxes between biochar and control plots.
401 Studies from temperate forest soils have also indicated that increases in soil CO_2 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^{-1} 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^{-1} 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^{-1} biochar increased soil pH but the biochar amount of 5
435 t ha^{-1} had no effect. Similarly, Noyce et al. (2015) found that the addition of 5 t ha^{-1} 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^{-1} yr^{-1} 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^{-1} yr^{-1}) 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 ≤ 0.5 kg ha^{-1} yr^{-1} in early successional forests (Zackrisson et al. 2004; DeLuca et
473 al. 2007). Furthermore, the rather high N deposition (7.4 kg ha^{-1} yr^{-1}) 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^{-1} yr^{-1} 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^{-1} 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^{-1} did not have a clear and
508 consistent effect on soil CO_2 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, Apaydın-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_2O 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_2) fixation in natural ecosystems. Glob Biogeochem Cycles 13:
571 623–645.
572 Clough TJ, Condron 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_2-fixing cyanobacteria. J Exp Bot 56(422):
593 3121–3127.

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

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

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

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

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

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

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

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

IUSS Working Group WRB (2015) World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil 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_2 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_2O and
628 CO_2 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, Tiirola 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_2-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, Ruysschaert 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_2 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 (±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^2	N g m^2	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 ± 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 μs cm^{-1}	1361 (226)	1462 (179)
Al mg g^{-1}	21.83 (1.68)	20.78 (1.07)
Ca mg g^{-1}	190.68 (10.34)	154.15 (7.17)
Cu mg g^{-1}	0.22 (0.01)	0.20 (0.01)
Fe mg g^{-1}	24.01 (2.09)	21.32 (1.10)
K mg g^{-1}	29.81 (3.27)	42.87 (6.73)
Mg mg g^{-1}	17.08 (1.09)	19.32 (1.55)
Mn mg g^{-1}	3.86 (0.16)	2.95 (0.10)
Na mg g^{-1}	3.30 (0.96)	2.71 (0.24)
Ni mg g^{-1}	0.07 (0.01)	0.08 (0.01)
P mg g^{-1}	10.78 (0.40)	7.41 (0.21)
S mg g^{-1}	5.61 (0.25)	4.33 (0.12)
Si mg g^{-1}	2.29 (0.11)	2.19 (0.05)
Zn mg g^{-1}	0.42 (0.01)	0.34 (0.01)

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751 Table 3. Monthly mean (±SE) soil temperatures (°C) at 5 cm depth in different treatments. The same
752 letters indicate no statistically significant differences among treatments.

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

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Table 4. The average biomass (± SE) of mosses (kg ha^{-1}) in different treatments.

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

Figure captions

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

Figure 2. Mean (±SE) soil CO_2 effluxes in different treatments in June and July. Different letters indicate statistically significant differences ($P < 0.05$) between treatments.

Figure 3. Mean (±SE) soil microbial biomass carbon, soil microbial biomass nitrogen and soil microbial biomass carbon:nitrogen ratio in control plots and 5 t ha^{-1} and 10 t ha^{-1} biochar (pyrolysis temperature 650°C) treatments. Different letters indicate statistically significant differences ($P < 0.05$) between treatments.

Figure 4. The mean (±SE) biological nitrogen fixation rates (µg m^{-2} d^{-1}) in each incubation temperature (10°C, 15°C and 20°C) in control plots and 5 t ha^{-1} and 10 t ha^{-1} biochar (pyrolysis temperature 650°C) treatments. Statistically significant differences ($P < 0.05$) between treatments in each temperature group are indicated by different lower-case letters, whereas statistically significant differences ($P < 0.05$) between temperatures are indicated by upper-case letters in parenthesis.

Figure 5. The mean (±SE) soil pH in the organic layer and upper 10 cm mineral soil layer in control plots and 5 t ha^{-1} and 10 t ha^{-1} biochar (pyrolysis temperature 650°C) treatments. Different letters indicate statistically significant differences ($P < 0.05$) between treatments.

Figure 6. The mean (±SE) net nitrogen mineralization, ammonification and nitrification rates in the organic layer and upper 10 cm mineral soil layer in control plots and 5 t ha^{-1} and 10 t ha^{-1} biochar (pyrolysis temperature 650°C) treatments. Different letters indicate statistically significant differences ($P < 0.05$) between treatments.

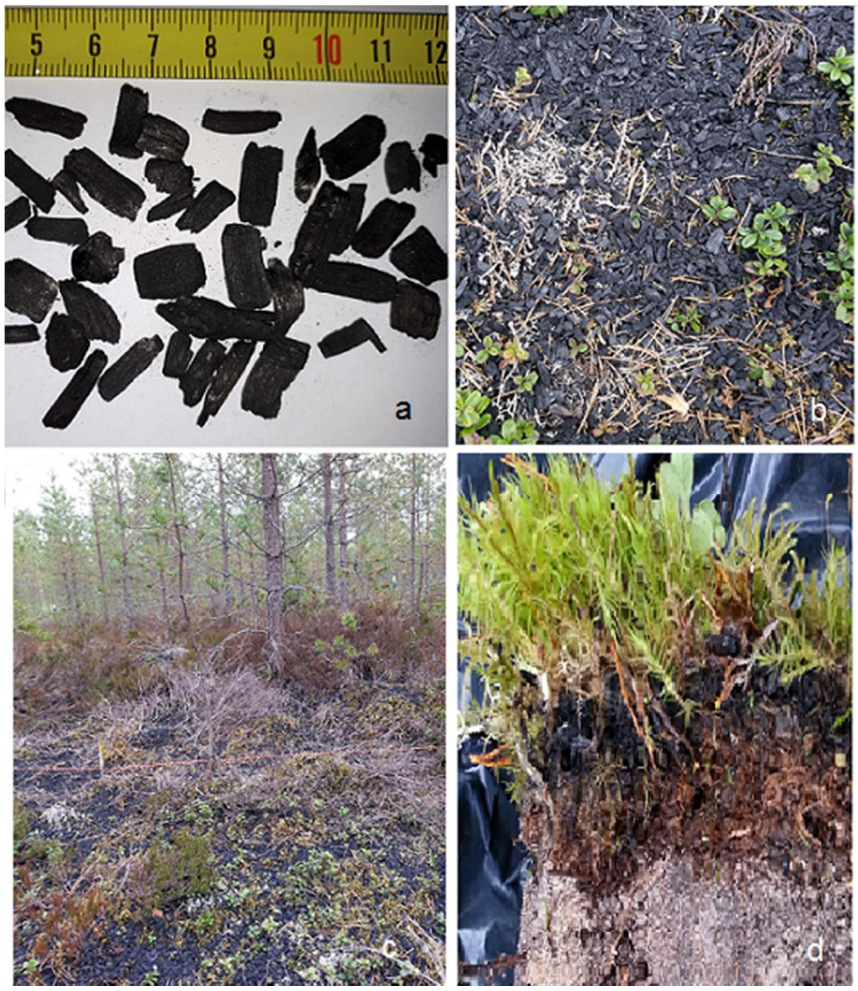


Fig. 1

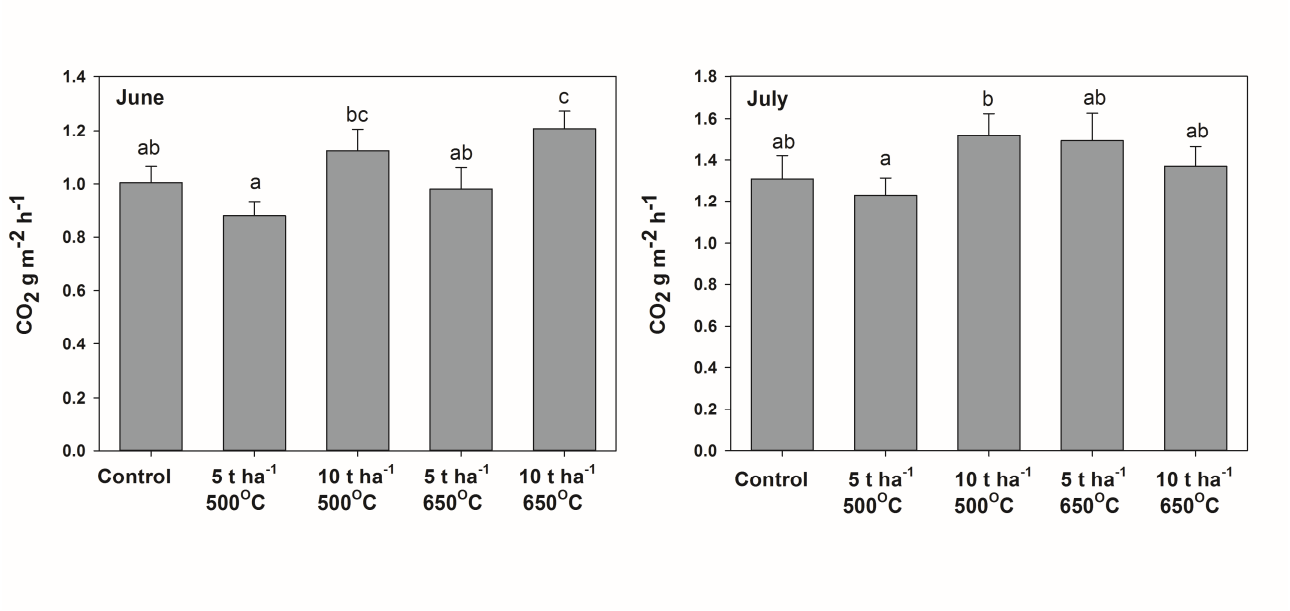
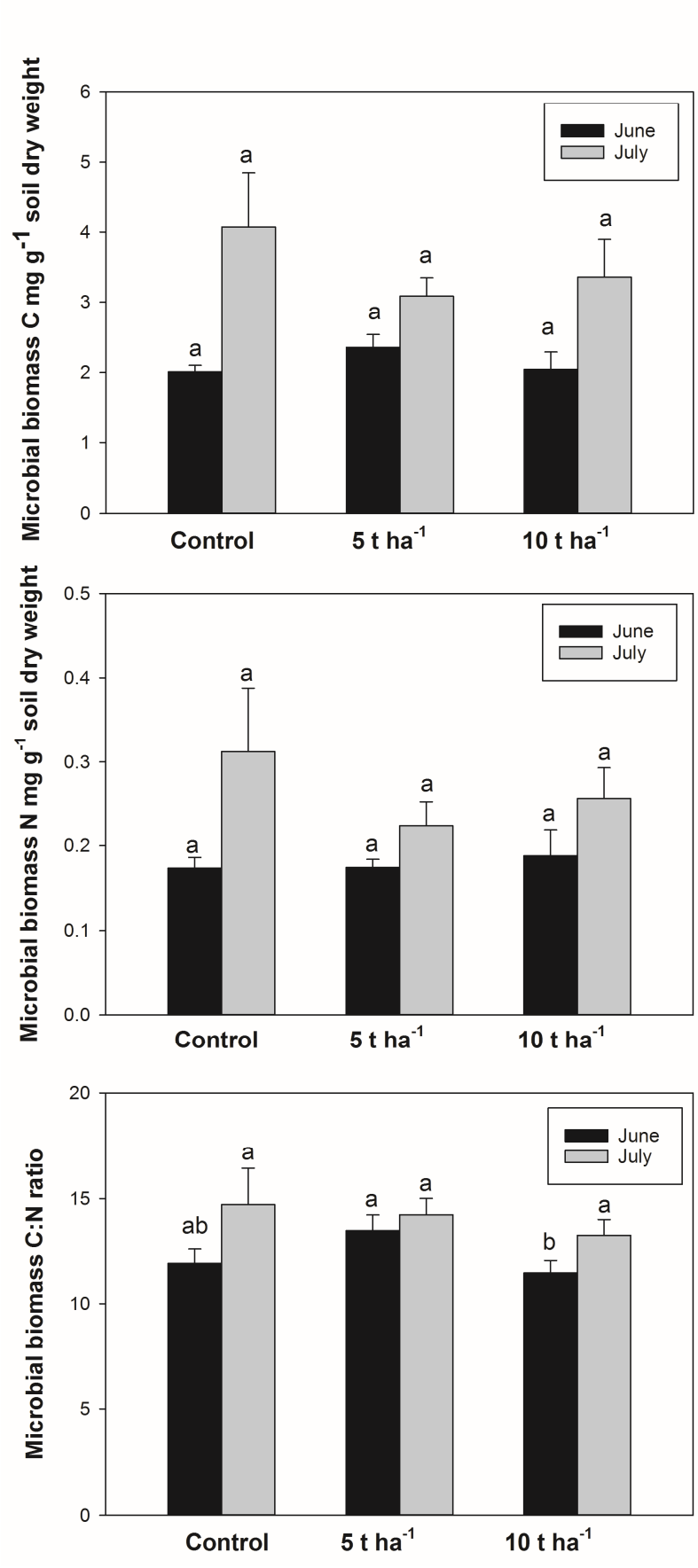
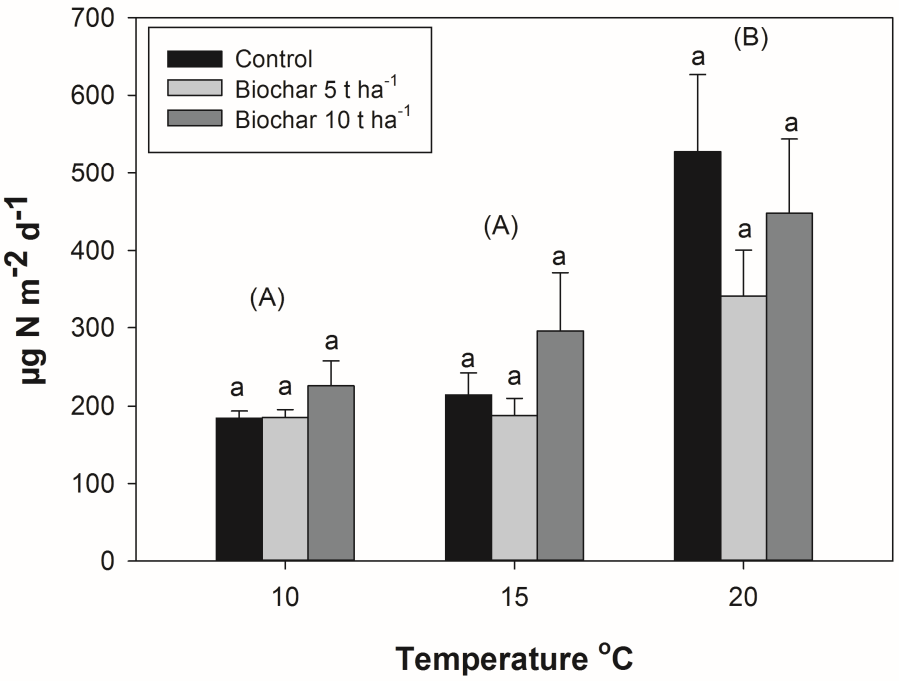

June
July
CO_2 g m^{-2} h^{-1}
ab
a
bc
ab
c
b
ab
Control
5 t ha^{-1} 500°C
10 t ha^{-1} 500°C
5 t ha^{-1} 650°C
10 t ha^{-1} 650°C

Fig. 2


Microbial biomass C mg g-1 soil dry weight
June
July
a
a
a
a
a
a
Control
5 t ha-1
10 t ha-1
Microbial biomass N mg g-1 soil dry weight
June
July
a
a
a
a
a
a
Control
5 t ha-1
10 t ha-1
Microbial biomass C:N ratio
June
July
ab
a
a
a
b
a
Control
5 t ha-1
10 t ha-1

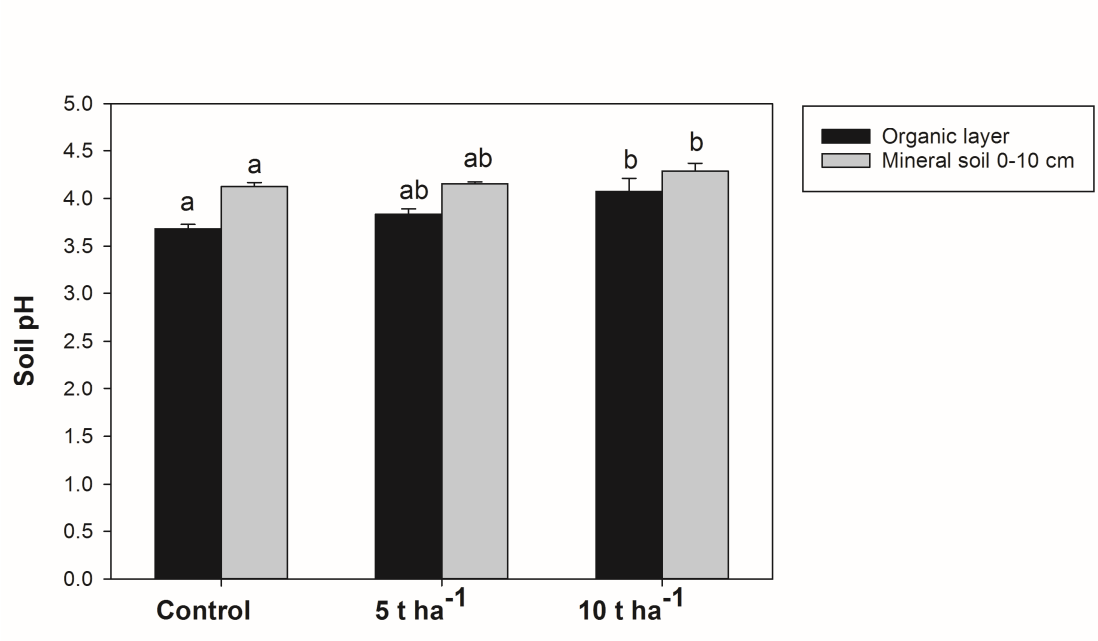
790

791 Fig.3


Control
Biochar 5 t ha-1
Biochar 10 t ha-1
µg N m-2 d-1
Temperature °C
(A)
(A)
(B)
a
a
a
a
a
a
a
a
a
0
100
200
300
400
500
600
700
10
15
20

792

793 Fig. 4



794

795 Fig. 5

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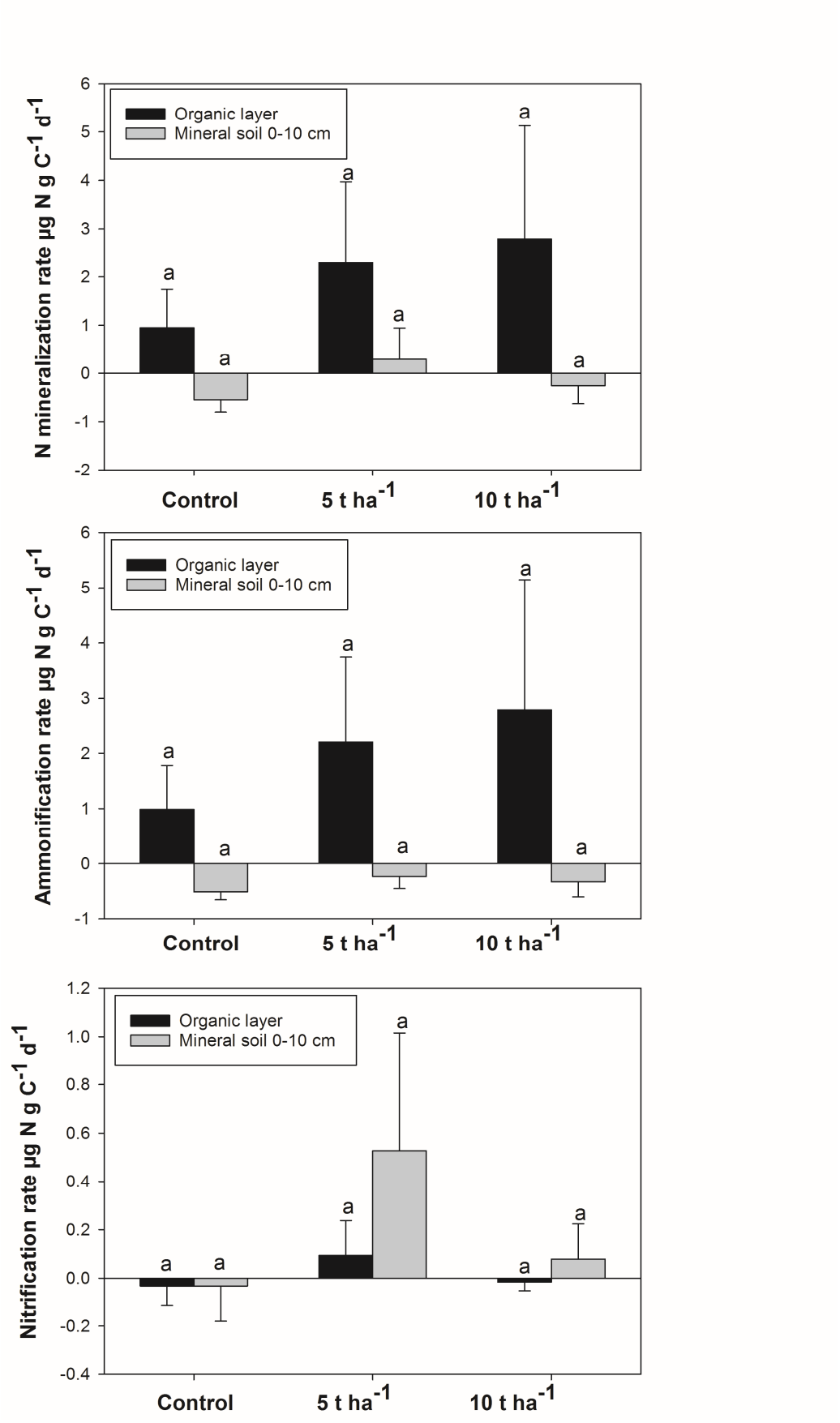
N mineralization rate µg N g C-1 d-1
Ammonification rate µg N g C-1 d-1
Nitrification rate µg N g C-1 d-1
Organic layer
Mineral soil 0-10 cm
Control
5 t ha-1
10 t ha-1
a

797

798 Fig. 6