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RESEARCH PAPER

Short-term effects of biochar on soil CO_2 efflux in boreal Scots pine forests

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Abstract

• ***Key message*** **During the first summer, wood biochar amendments increased soil temperature, pH, and soil CO_2 effluxes in a xeric boreal Scots pine forest. The increase of soil CO_2 efflux could be largely explained by increases in by soil temperature. Higher biochar application rates (1.0 vs 0.5 kg m^{-2}) led to higher soil CO_2 efflux while the pyrolysis temperature of biochar (500 or 650 °C) had no effect on soil CO_2 efflux.**

• ***Context*** Using biochar as a soil amendment has been proposed to increase the carbon sequestration in soils. However, a more rapid soil organic matter turnover after biochar application might reduce the effectiveness of biochar applications for carbon sequestration. By raising the pyrolysis temperature, biochar with lower contents of labile carbohydrates can be produced.

• ***Aims*** To better understand the effects of biochar on boreal forest soil, we applied two spruce biochar with different pyrolysis temperatures (500 °C and 650 °C) at amounts of 1.0 and 0.5 kg m^{-2} in a young xeric Scots pine forest in southern Finland.

• ***Methods*** Soil CO_2, microbial biomass, and physiochemical properties were measured to track changes after biochar application during the first summer.

• ***Results*** Soil CO_2 increased 14.3% in 1.0 kg m^{-2} treatments and 4.6% in 0.5 kg m^{-2}. Soil temperature and pH were obviously higher in the 1.0 kg m^{-2} treatments. Differences in soil CO_2 among treatments disappear after correcting by soil temperature and soil moisture.

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• ***Conclusion*** Biochar increased soil CO_2 mainly by raising soil temperature in the short term. Higher biochar application rates led to higher soil CO_2 effluxes. The increase in soil CO_2 efflux may be transient. More studies are needed to get the optimum biochar amount for carbon sequestration in boreal forest.

Keywords Biochar · Soil microbial biomass · Soil CO_2 · Soil temperature · Boreal forest

1 Introduction

Biochar is a C-rich material produced by pyrolyzing biomass or other organic materials, such as agricultural crop residues, wood, and green waste in an oxygen-depleted environment (Ahmed and Schoenau 2015). In 2001, the term "biochar" was coined after Glaeser describing "Terra preta" soils (Glaser et al. 2001). It is used as a soil amendment to increase productivity, restore soil fertility, sequester C in soil, and reduce atmospheric CO_2 concentration (Woolf 2008; Van Zwieten et al. 2010; Wang et al. 2014). Its aromatic structures make it resistant to microbial decomposition (Kumar et al. 2005; Schimmelpfennig and Glaser 2012). Since that, the majority of studies on the effects of biochar application on soils have been on agricultural soils (Prayogo et al. 2014; Lu et al. 2014; Zhang et al. 2017). Contrary to agricultural ecosystems, the effects of newly added biochar on forest ecosystems are still uncertain.

Previous studies on the effects of biochar addition on soil CO_2 effluxes in subtropical and temperate forests show inconsistent results. Some studies show that CO_2 fluxes increase (Mitchell et al. 2015; Bamminger et al. 2016; Hawthorne et al. 2017; Johnson et al. 2017); others indicate a decrease in fluxes; and some do not show any effect of biochar. Studies on the effects of large-scale biochar application in boreal forests are rare.

Biochar increase N uptake by some plant species (Wardle et al. 1998), increase nutrient availability (Glaser et al. 2001), and enhance humus formation (Glaser et al. 2001). Therefore, it may mitigate the negative effects of biomass harvesting in N-limited boreal forests. Biochar can also act as a soil conditioner; it enhances plant growth by improving soil aggregation, soil porosity, cation-exchange capacity, and pH (Biederman and Harpole 2013; Thomas and Gale 2015; Li et al. 2018) and by adsorbing toxic compounds (Wardle et al. 1998; Robertson et al. 2012). These changes in soil physiochemical properties may affect soil CO_2 emissions (Peng and Thomas 2010) by altering soil microbial diversity and activity and by changing fine root production and root respiration (He et al. 2017). Moreover, previous studies have found that the labile C fractions of biochar may accelerate the decomposition of native soil organic matter in a process known as the "positive priming effect" (Luo et al. 2013; Maestrini et al. 2015; Fang et al. 2015; Wang et al. 2016). In addition, Zackrisson et al. (1996) and Wardle et al. (1998, 2008) indicated that fire-derived charcoal can adsorb phenolic compounds and accelerate organic matter decomposition in boreal forests. On the other hand, a negative priming effect can also occur due to the fact that biochar can (i) absorb labile C, reducing its availability to soil microorganisms (Jones et al. 2012), (ii) absorb enzymes involved in the decomposition of soil organic matter and thus decrease their activity (Woolf and Lehmann 2012), and (iii) directly absorb soil CO_2 (Li et al. 2018).

An increase in soil CO_2 efflux at the initial stage after biochar incorporation has been reported, which may be due to the rapid decomposition of the labile component of C in the biochar (Cross and Sohi 2011; Luo et al. 2011; Ouyang et al. 2014), accelerated decomposition of native soil C induced by the biochar (Singh and Cowie 2015; Bruckman et al. 2015; Wang et al. 2016), or the increasing soil temperature (biochar addition decreases the soil surface albedo) (Genesio et al. 2012). These impacts of biochar addition on soil depend on soil properties (Kolb et al. 2009; Spokas et al. 2009), vegetation, and local environmental and climatic conditions (He et al. 2018). Biochar-induced increases in soil CO_2 fluxes have been shown to increase with latitude and have been attributed to increased soil temperature after biochar addition and the larger stimulation of microbial activity in high-latitude, temperature-limited ecosystems (He et al. 2017). In addition, different feedstocks and pyrolysis temperatures will result in differences of biochar physical and chemical properties. In general, wood biochar increases soil CO_2 efflux to a lesser degree compared with other types of biochar (Zimmerman et al. 2011; He et al. 2017). Furthermore, by raising the pyrolysis temperature, biochar with none or low contents of unconverted cellulosic and hemicellulosic fractions can be produced, as these labile carbohydrates are rapidly mineralized; their presence lowers the biochar-C sequestration potential (Bruun et al. 2011). Ameloot et al. (2013) found CO_2 emissions were significantly higher in 350 °C biochar treatments than control, while no significant difference in 700 °C biochar treatments. Fang et al. (2015) showed that 550 °C wood biochar was more effective for long-term soil C storage relative to 450 °C wood biochar. Meanwhile, the dosage of biochar may also affect the responses in soil respiration. Previous studies have shown increased soil CO_2 effluxes with increasing biochar application rates in temperate forests (Mitchell et al. 2015), while meta-analyses from agricultural soils have only shown increases of soil CO_2 effluxes at high (2–4 kg m^{-2}) amendment rates (Song et al. 2016; He et al. 2017). In boreal forests, the effects of biochar on soil CO_2 efflux are rarely studied. Gundale et al. (2016) applied 1-kg m^{-2} biochar to soil by mixing it into the soil after clear-cutting and stump harvesting and observed no changes in soil respiration suggesting its good stability against decomposition. Due to increasing concern for carbon loss, impacts on water and biodiversity, less invasive forest regeneration practices are getting more attention as

an alternative to clear-cutting which has so far been the most common method of forest harvesting in boreal forests. In the future, the harvesting techniques where part of the trees are left standing and the soil surface is not mixed after the harvesting are probably becoming more common. However, there is little information on the effects of biochar treatments on intact forest soils in boreal forests.

In this study, commercially available biochar produced from Norway spruce (*Picea abies* (L.) H. Karst) woodchips by controlled pyrolysis at 500 °C and 650 °C was used at two typically and economically feasible amounts (0.5 and 1.0 kg m^{-2}) on intact forest soil (see e.g. Bruckman et al. 2015; Gundale et al. 2016). The aim of this study was to determine (1) whether biochar addition changes soil respiration, soil temperature, soil moisture, soil pH, and microbial biomass during the initial 5 months after application on boreal Scots pine (*Pinus sylvestris* L.) forest stands; and (2) whether the pyrolysis temperature and amount of biochar affect these aforementioned factors. We hypothesized that biochar amendment will increase soil CO_2 efflux by changing the soil physiochemical and biological properties at the initial stage and higher efflux is expected at higher biochar application rates (1 vs 0.5 kg m^{-2}). Besides, as biochar with lower contents of labile carbohydrates can be produced by rising the pyrolysis temperature, lower soil CO_2 efflux is anticipated when biochar pyrolyzed at higher temperatures (650 °C vs 500 °C) is applied to soil.

2 Material and methods

2.1 Site description

The field experiment was performed during the summer of 2015 in Juupajoki (61° 48′ N and 24° 18′ E, 181 m a.s.l.) close to the Hyytiälä Forestry Field Station in southern Finland. The experiment was established in young approximately 20-year-old Scots pine (*Pinus sylvestris* L.) forest stands that were naturally regenerated from seed trees after harvesting. These trees belong to the *Vaccinium*- and *Calluna*-type forests according to the Finnish site–type classification (Cajander 1949). All plots are common low-fertility xeric site types. *Vaccinium vitis-idaea* L., *Calluna vulgaris* (L.) Hull., *Empetrum nigrum* L., and *V. myrtillus* L. were the dominant species of the understorey vegetation. The forest floor was covered with mosses (*Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) Schimp.) and some lichens (*Cladina* sp.). The soil is a nutrient-poor, well-drained haplic podzol (IUSS Working Group WRB, FAO 2015), and the soil texture is coarse sand (Table 1). The long-term (1981–2010) mean annual air temperature in the area is 3.5 °C, the mean annual precipitation is 700 mm, and the snow cover duration is 145–160 days (Pirinen et al. 2012). During the experimental period (May–September 2015), the mean temperature of the area was + 12.1 °C; May was the coldest month (mean + 8.6 °C) and August was the warmest (mean + 16.2 °C). The mean monthly precipitation during the experiment was 67 mm. Mean precipitation was highest in July (118 mm) and lowest in August (18 mm). The main characteristics of study plots in May before biochar application are shown in Table 1.

2.2 Experimental design and measurement

2.2.1 Experimental setup

The experiment was set up as a randomized block design with four replicates (called blocks) and five plots (15 m × 15 m rectangles) within each block (Zhu et al. 2020). To avoid pseudoreplication, blocks were separated by a few hundred meters from each other and belong to four different forest stands within a radius of 1.5 km. The terrain of the blocks is flat with no slope. Within each block, we delimited suitable homogenous areas. The distance between each plot was 10 m, and a 2.5-m wide buffer zone surrounding plot edges was not used for measurements. Treatments were assigned randomly to plots in each block. Biochar (hereafter BC500 and BC650) used in the study were produced from Norway spruce (*Picea abies* (L.) H. Karst) wood chips at 500 °C and 650 °C, respectively (manufactured by Sonnenerde GmbH, Riedlingsdorf, Austria). The grain size was 5–10 mm. Both types of biochar were applied on the plots at two different rates (0.5 kg m^{-2} and 1.0 kg m^{-2}). For simplicity, we will use abbreviated treatment names; e.g., T500M1.0 will denote plots that were amended with biochar produced at 500 °C at a rate of 1.0 kg m^2. Thus, the five treatments were T500M0.5, T500M1.0, T650M0.5, T650M1.0, and control (without biochar amendment). The biochar was spread manually on top of the humus layer (0.5–2.0 cm) in May 2015 to avoid soil disturbance and damage to roots. During our experimental period, biochar remained on the surface of soil, but the moss-dominated vegetation did not suffer from biochar addition and remained stable. The mosses did not cover the biochar until the second summer after treatment (Palviainen et al. 2018).

2.2.2 Soil and biochar physicochemical properties

Soil samples were collected from the organic layer and the upper 15 cm of the mineral soil layer using a stainless soil corer (diameter 5.5 cm) at nine randomly selected locations in each treatment in May, August, and September. The samples were divided into subsamples for the organic layer and mineral soil depths of 0–5 cm and 5–15 cm. To determine the chemical characteristics of the biochar, two biochar samples were collected from four biochar bags used for transporting the biochar from the factory to the experimental sites. Prior to analysis, soil and biochar samples were dried (60 °C, 24 h) and ground; soil was sieved through a 2-mm sieve to remove visible stones, coarse roots, and leaves before grinding. Subsamples were dried at 105 °C to determine the dry mass of the sample. The C and N concentrations of soil and biochar were analyzed from the homogenized samples with

Table 1 Main characteristics of treatment plots before biochar application in May. T500M1.0 denotes plots that were amended with biochar produced at 500 °C at a rate of 1.0 kg m^2; T500M0.5 denotes plots that were amended with biochar produced at 500 °C at a rate of 0.5 kg m^2; T650M1.0 denotes plots that were amended with biochar produced at 650 °C at a rate of 1.0 kg m^2; T650M0.5 denotes plots that were amended with biochar produced at 650 °C at a rate of 0.5 kg m^2; and control denotes plots without biochar amendment. Comparison among treatment plots was done by one-way ANOVA. The same letters indicate no statistically significant differences among treatments ($n = 4$, $P < 0.05$). Values are mean ± SE

	T500M0.5	T500M1.0	T650M0.5	T650M1.0	Control
Number of trees (height > 1.3 m) per hectare	4950	3825	3975	3250	4125
Mean tree height (m)	4.72 (0.09) a	4.94 (0.10) a	5.17 (0.18) a	5.06 (0.20) a	5.22 (0.11) a
Mean diameter at breast height (cm)	4.58 (2.09) a	4.88 (1.88) a	4.35 (2.63) a	4.33 (2.20) a	4.45 (2.95) a
Leaf area index (LAI)	2.57 (0.55) a	2.54 (0.33) a	2.20 (0.9) a	2.27 (0.31) a	2.20 (0.62) a
N (%) in soil organic layer	1.01 (0.19) a	0.83 (0.22) a	1.09 (0.30) a	0.63 (0.37) a	1.14 (0.31) a
N (%) in mineral soil 0–5 cm	0.08 (0.02) a	0.11 (0.02) a	0.11 (0.04) a	0.08 (0.03) a	0.12 (0.09) a
N (%) in mineral soil 5–15 cm	0.07 (0.01) a	0.06 (0.03) a	0.06 (0.00) a	0.06 (0.00) a	0.05 (0.01) a
C (%) in soil organic layer	33.00 (7.10) a	30.07 (10.85) a	33.08 (8.07) a	22.88 (15.53) b	36.62 (6.65) a
C (%) in mineral soil 0–5 cm	2.22 (0.33) a	2.75 (0.80) a	3.00 (1.75) a	2.18 (0.93) a	3.03 (1.73) a
C (%) in mineral soil 5–15 cm	1.21 (0.06) a	1.05 (0.68) a	1.25 (0.25) a	1.21 (0.17) a	0.99 (0.18) a
C/N in soil organic layer	32.56 (3.23) a	35.50 (5.00) a	30.56 (1.90) a	34.08 (6.42) a	32.91 (5.42) a
C/N in mineral soil 0–5 cm	28 (3.43) a	24.06 (2.29) a	24.82 (6.03) a	27.56 (9.09) a	26.80 (4.42) a
C/N in mineral soil 5–15 cm	18.40 (4.6) a	18.09 (2.34) a	19.59 (3.20) a	20.59 (2.19) a	18.97 (0.35) a
pH	4.49 (0.32) a	4.54 (0.24) a	4.48 (0.44) a	4.44 (0.17) a	4.64 (0.34) a
Bulk density (g/cm^3)	0.53 (0.08) a	0.56 (0.09) a	0.51 (0.11) a	0.52 (0.09) a	0.51 (0.12) a
Electric conductivity (μs/cm)	41.88 (13.56) a	39.13 (15.64) a	40.14 (15.14) a	42.50 (13.60) a	38.13 (15.79) a
Silt (%) in mineral soil 0–5 cm	18.43	12.94	15.80	16.75	13.62
Sand (%) in mineral soil 0–5 cm	81.57	87.06	84.2	83.25	86.38
Silt (%) in mineral soil 5–15 cm	12.08	10.52	14.63	14.93	11.23
Sand (%) in mineral soil 5–15 cm	87.92	89.48	85.37	85.07	88.77

an elemental analyzer (VarioMax CN, Elementar Analysen Systeme GmbH, Hanau, Germany). PH were determined using a pH meter (PHM210, Radiometer Analytical, France) on a 1:2.5 (v:v) sample/water solution. Electric conductivity (EC) was measured by an electric meter (JENWAY 4010 Conductivity, TER Calibration Ltd., Wigan, UK). Soil particle size distribution was determined by a Coulter LS device (LS230, Coulter Corp., Miami, FL, America). The loss on ignition (LOI) of biochar was determined by combusting samples at 550 °C for 3 h. The concentrations of P, K, Ca, Mg, S, Fe, Al, Na, Cu, Mn, Ni, Si, and Zn in the biochar were determined by an ICP atomic emission spectrophotometer (ARL 3580 OES, Fison Instruments, Valencia, USA). Characteristics of soil and biochar are presented in Tables 1 and 2 (Palviainen et al. 2018), respectively.

2.2.3 Soil microbial biomass

Nine soil samples from per plot were collected for microbial biomass C and N analysis in May, August, and September. Samples were taken from the humus layer using a stainless soil corer (diameter 10 cm) close to those sampling points where the soil samples were taken for the soil analyses. The samples were put into 45-mL plastic containers and kept in an ice box before storage at − 20 °C. Prior to analysis, soil samples were incubated at 4 °C for 7–9 days. The nine samples from each subplot were pooled into three samples after removing visible stones, coarse roots, and leaves and sieving through a 2-mm sieve. Soil microbial biomass C and N were measured by a slightly modified chloroform fumigation extraction method (Joergensen 1996). Three grams of fresh soil (diameter < 2 mm) from each sample was weighed and placed into glass beakers, then fumigated with 50 mL ethanol-free chloroform ($CHCl_3$) in a vacuum desiccator. Another sample of 3 g was placed in plastic bottles in another desiccator as non-fumigated control samples. Both desiccators were kept at 25 °C in the dark for 24 h. After fumigation, 0.5 M potassium sulfate (K_2SO_4) was used to extract the fumigated and non-fumigated samples (oven-dry basis soil: K_2SO_4 = 1:20). Then, the samples were shaken at 200 RPM for 1 h and filtered through Whatman no. 42 filter papers. The filtrate was then used to analyze the microbial C and N by a TOC-V_{CPH} analyzer (Shimadzu Corp., Kyoto, Japan). The microbial biomass C and N (mg g^{-1}) were calculated as:

$$\text{Microbial biomass C/N} = \frac{\left(\frac{F \cdot (V1/1000)}{M1} - \frac{UF \cdot (V2/1000)}{M2}\right)}{K} \quad (1)$$

Table 2 Comparison of three linear mixed models with the base model. *T* is treatment, *M* is month, *B* is block, *C* is the collar in plot, R_{May} is soil CO_2 efflux in May (prior to treatments) as covariate, *Tem* is soil temperature, *Moi* is soil moisture, and *ln(R)* is natural logarithm of soil CO_2; *a* denotes the coefficients of fixed effects and α denotes the coefficients for random factors; $\mathcal{E}$ is the error term. Equation 5 tests if biochar application increases the soil CO_2 efflux while Eqs. 6–8 test if soil CO_2 efflux differs at a given value of soil moisture and soil temperature. Comparison among models was done by the chi-squared test. *P* value means the difference between each linear mixed model and the base model

Model	AIC	BIC	LogLik	Chi-square	*P* value
Equation 5: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + \propto_1 B + \propto_2 C + \varepsilon$	777.47	827.53	−376.74	NA	NA
Equation 6: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Moi} + \propto_1 B + \propto_2 C + \varepsilon$	774.22	845.14	−370.11	0.00	> 0.05
Equation 7: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Tem} + \propto_1 B + \propto_2 C + \varepsilon$	684.12	755.04	−325.06	103.35	< 0.05
Equation 8: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Tem} + a_6\text{Moi} + \propto_1 B + \propto_2 C + \varepsilon$	647.72	739.50	−301.86	136.50	< 0.05

where *F* (mg L^{-1}) is the total organic C or total N concentration of a fumigated sample, *V*1 (mL) is the volume of K_2SO_4 added to extract a fumigated sample, 1000 is the unit conversion factor (for converting mL into L), *M*1 (g) is the dry mass of a fumigated sample, *UF* (mg L^{-1}) is the total organic C or total N concentration of a non-fumigated sample, *V*2 (mL) is the volume of K_2SO_4 added to extract a non-fumigated sample, *M*2 (g) is the dry mass of a non-fumigated sample, and *K* is the soil-specific calibration factor set to 0.45 for C (Beck et al. 1997) or 0.54 for N (Brookes et al. 1985).

2.2.4 Soil respiration

The collars (diameter 22 cm) for soil CO_2 efflux measurements were inserted permanently at 0.02-m depth in the mor layer above the rooting zone to avoid damaging the roots and were sealed with a thin layer of sand placed around both the inner and outer sides of the collars to prevent leakage during flux measurements. Ground vegetation inside the collars remained intact. Six collars were distributed randomly in each of the 20 plots for soil respiration measurements. Thus, there were 24 collars in each treatment and 120 collars in total.

Soil CO_2 efflux was measured with a closed chamber system consisting of a darkened cylindrical polycarbonate chamber (diameter 20 cm, height 30 cm), a CO_2 analyzer, a sensor for relative humidity and temperature, and a data logger (Pumpanen et al. 2015). The chamber was placed onto the collars only during the measurements, which lasted 4 min. During the measurements, air inside the chamber was mixed continuously by a small fan. The CO_2 concentration inside the chamber was recorded with a GMP343 diffusion–type CO_2 probe (Vaisala Oyj, Vantaa, Finland) at 5-s 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 measured daily at the nearby (4 km away) Station for Measuring Ecosystem-Atmosphere Relations (SMEAR II) (Schobesberger et al. 2016). The CO_2 efflux was calculated as the slope of a linear regression of CO_2 concentration in the chamber against time. Only measurements taken between 45 s and 3 min after the closure were included in the fitting.

Soil temperature at a 5-cm depth was measured by a dual-input digital thermometer (Fluke-52-2, Fluke Corp.). Volumetric water content was measured by a ThetaProbe–type moisture meter simultaneously near the collar (HH2, Delta-T Devices Ltd., Cambridge, UK). The measurements were performed between 9:00 and 11:00 am on clear days at approximately the end of each month starting in May 2015 (before biochar amendment) and finishing in September 2015.

An exponential regression model was used to describe the relationship between soil CO_2 and soil temperature:

$$y = a \times \exp(kt) \tag{2}$$

where *y* is the soil respiration, *t* is the soil temperature at 5-cm depth, and *a* and *k* are the model coefficients. The temperature sensitivity, Q_{10}, was calculated from this model using the following equation (Liu et al. 2011; Luo et al. 2011; Song et al. 2013):

$$Q_{10} = \exp(10k) \tag{3}$$

2.3 Statistical analysis

The effect of biochar application on soil temperature, soil moisture, pH, EC, MBC, MBN, MBC/N, and soil CO_2 efflux were analyzed with linear mixed model followed by Fisher's least significant difference (LSD) test. Treatment and month were taken as fixed factors, and block and the collar were set as random factors. In soil CO_2 analysis, the soil CO_2 efflux in May of each collar was used as covariate, as this measurement preceded the biochar applications and collars were not moved during the experiments. This was done because soil CO_2 efflux varies between sites and collars. These pre-treatment values as covariates have been used to adjust the soil CO_2 effluxes for variation in soil properties within our experiment. The relationship between

soil CO_2 efflux and soil temperature and moisture among different treatments was tested by three different linear mixed models, and we compared them to the base model by the chi-squared test. The model comparison was performed with R version 3.5.3 using *stats* package (R Core Team 2019). We used mixed models for the analysis of our results since they provide a more flexible approach for nested repeated measures designs as in our experiment. Our analysis follows the approach suggested by Cleophas and Zwinderman (2012) in chapter 55 as well as the approach by Kulmala et al. (2014).

Differences in soil temperature, soil moisture, pH, EC, MBC, MBN, and MBC/N among treatments were estimated by a linear mixed model (Eq. 4):

$$Y_i = a_{1+}a_2\ T + a_3\ M + \propto_1 B + \propto_2 C + \varepsilon_i \quad (4)$$

where Y_i is measured value of the observation i of the respective environmental variable (soil temperature, moisture, pH, EC, MBC, MBN, and MBC/N). T is treatment, M is month, B is block, C is the collar in plot, a denotes the coefficients of fixed effects and α denotes the coefficients for random factors, and $\mathcal{E}$ is the error term. All statistical analyses were performed using IBM SPSS Statistics 23.0 (IBM Crop., Armonk, NY). Differences were considered statistically significant when P value was < 0.05.

The base model (Eq. 5) estimates differences of soil CO_2 efflux among treatments without accounting for differences in the physical environment:

$$ln(R) = a_1 + a_2T + a_3M + a_4R_{\mathrm{May}} + \propto_1 B + \propto_2 C + \varepsilon \quad (5)$$

where $ln(R)$ is the natural logarithm of soil CO_2 and R_{May} is soil CO_2 efflux in May (prior to treatments) as covariate.

The linear mixed models estimate differences of soil CO_2 efflux among treatments at a given value of soil moisture (Eq. 6) or soil temperature (Eq. 7) or both (Eq. 8):

$$ln(R) = a_1 + a_2T + a_3M + a_4R_{\mathrm{May}} + a_5\mathrm{Moi} + \propto_1 B + \propto_2 C + \varepsilon \quad (6)$$

$$ln(R) = a_1 + a_2T + a_3M + a_4R_{\mathrm{May}} + a_5\mathrm{Tem} + \propto_1 B + \propto_2 C + \varepsilon \quad (7)$$

$$ln(R) = a_1 + a_2T + a_3M + a_4R_{\mathrm{May}} + a_5\mathrm{Tem} + a_6\mathrm{Moi} + \propto_1 B + \propto_2 C + \varepsilon \quad (8)$$

where Tem is soil temperature and Moi is soil moisture. We emphasize that the hypotheses behind Eq. 5 and Eqs. 6–8 are different. Equation 5 tests if biochar application increases the soil CO_2 efflux while Eqs. 6–8 test if soil CO_2 efflux differs at a given value of soil moisture and soil temperature.

3 Results

3.1 Physicochemical environment in different treatments

3.1.1 Soil temperature and moisture dynamics in different treatments

The mean soil temperatures in biochar treatments T500M0.5, T500M1.0, and T650M1.0 were significantly higher ($P < 0.05$) compared with those in the control (Fig. 1a). The mean soil temperature was highest in T650M1.0 (12.1 °C) and lowest in the control (11.4 °C) (Table 4). The amount of BC650 affected soil temperature significantly. Soil temperature in all treatments increased from May to August (the warmest month) and then dropped in September but remained higher than in May (Fig. 3a).

Responses of soil moisture to biochar application were not consistent. The mean soil moisture increased significantly in T500M0.5 ($P < 0.05$) and decreased significantly in T500M1.0 ($P < 0.05$) compared with that in control (Fig. 1b). The mean soil moisture was highest in T500M0.5 (0.14 $m^3\ m^{-3}$) and lowest in T500M1.0 (0.11 $m^3\ m^{-3}$) (Fig. 1b). Soil moisture with each treatment changed only slightly between May and July, then decreased sharply in August (the driest month) and increased again in September (Fig. 3b).

3.1.2 Soil pH and EC dynamics in different treatments

T500M1.0, T650M0.5, and T650M1.0 showed significant increases in soil pH ($P < 0.05$). The highest mean pH was measured in T500M1.0 (pH = 3.71) and the lowest in the control (pH = 3.55). The pH in treatments with 1.0 kg m^{-2} biochar was always significantly higher than in those with 0.5 kg m^{-2} biochar ($P < 0.05$) (Fig. 1c, Table 4).

Electric conductivity (EC) was highest in T500M0.5 (183.6 μs cm^{-1}) and lowest in control (168.9 μs cm^{-1}). The average increase of EC was 12.2 μs cm^{-1} and 5.4 μs cm^{-1} in 0.5 kg m^{-2} and 1 kg m^{-2} biochar treatments, respectively (Fig. 1d, Table 4).

3.2 Dynamics of C and N in microbial biomass in different treatments

The difference in the amount of C and N in the microbial biomass among the treatments was not statistically significant ($P > 0.05$) (Fig. 2a, b). The C/N ratios of microbial biomass were lower for biochar treatments (8.43) compared with those for the control (8.86); the difference was not statistically significant ($P > 0.05$) (Fig. 2c). The microbial biomass showed a clear seasonal pattern and increased over the growing season. The C/N ratio decreased during the same time. Biochar did not have any effect on the seasonal dynamics, and there was no

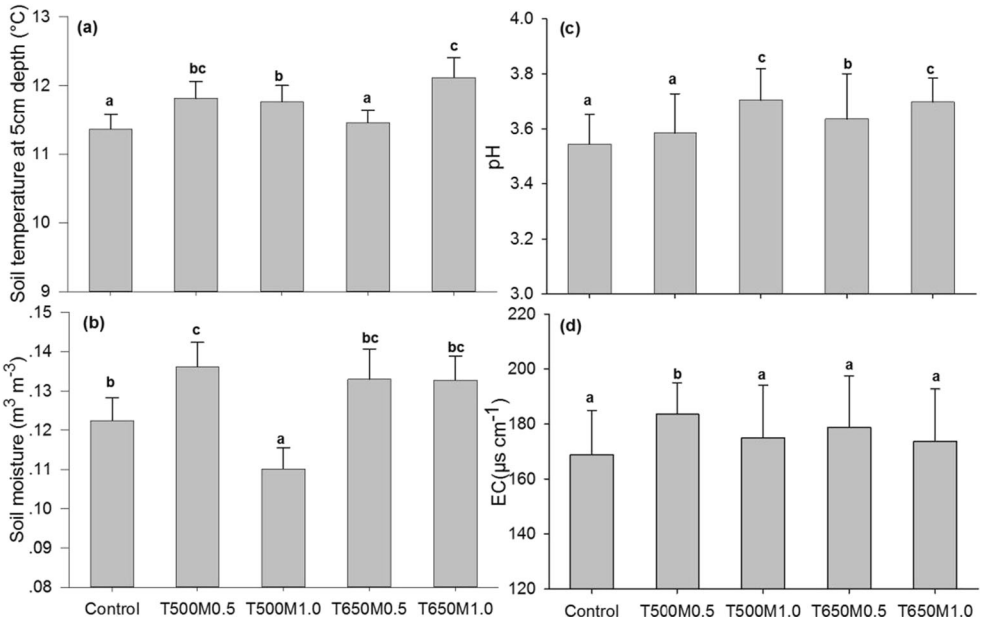

Fig. 1 Mean (± SE) soil temperature at 5-cm depth (**a**) and mean soil moisture (**b**) from June to September and mean soil pH (**c**) and mean electric conductivity (**d**) of August and September in different treatments (6 sample points in each block, 4 blocks for each treatment). T500M1.0 denotes plots that were amended with biochar produced at 500 °C at a rate of 1.0 kg m^2; T500M0.5 denotes plots that were amended with biochar produced at 500 °C at a rate of 0.5 kg m^2; T650M1.0 denotes plots that were amended with biochar produced at 650 °C at a rate of 1.0 kg m^2; T650M0.5 denotes plots that were amended with biochar produced at 650 °C at a rate of 0.5 kg m^2; and control denotes plots without biochar amendment. Each treatment had four replicates. Comparison among treatments was analyzed by linear mixed model with Fisher's least significant difference (LSD) test. Different letters indicate statistically significant differences ($P < 0.05$) between treatments

indication that biochar would change the dynamics of microbial biomass in our site ($P > 0.05$) (Fig. 3d–f).

3.3 Soil CO_2 efflux dynamics in different treatments

Mean soil CO_2 fluxes increased by 10 μg m^{-2} h^{-1} (for T500M0.5), 70 μg m^{-2} h^{-1} (T650M0.5), 132 μg m^{-2} h^{-1} (T650M1.0), and 156 μg m^{-2} h^{-1} (T500M1.0), lowest in the control (868 μg m^{-2} h^{-1}) and highest in T500M1.0 (1024 μg m^{-2} h^{-1}). Soil CO_2 fluxes increased 14.3% ($P < 0.05$) in 1.0 kg m^{-2} treatments and 4.6% ($P > 0.05$) in 0.5 kg m^{-2} treatments. Pyrolysis temperature of biochar did not affect soil CO_2 effluxes (Fig. 2d). Soil CO_2 efflux increased over time from May to July, then decreased (Fig. 3c, Appendix Table 4).

3.4 Comparison using linear mixed models

When no environmental factors were included in Eq. 5, 1.0 kg m^{-2} biochar treatments had significantly higher CO_2 effluxes than the control ($P < 0.05$). Table 2 shows that Eq. 6 (only taking soil moisture into account) performed only slightly ($P > 0.05$) better fit to the data compared with Eq. 5. Inclusion of temperature (Eq. 7) led to an obviously ($P < 0.05$) improved performance (as well as indicated by a large drop in the AIC values). The combined model (Eq. 8) had the lowest AIC; the treatment effects were not significant ($P > 0.05$) while effects of soil temperature and soil moisture were statistically significant ($P < 0.05$) (Table 3) Detail results of Eq. 8 shows the relationships between soil CO_2 fluxes and soil temperature and moisture among treatments in Appendix Table 5.

3.5 The relationships between soil physical properties and soil CO_2 efflux

An exponential relationship was found between soil temperature at 5 cm, soil moisture, and soil CO_2 effluxes. The R^2 was 0.23 for soil CO_2 efflux at high soil moisture (0.078–0.412 m^3 m^{-3}) and 0.09 at low soil moisture (0.008–0.076 m^3 m^{-3}). The Q_{10} values for high and low moisture conditions were 2.59 and 1.48, respectively (Fig. 4).

4 Discussion

Few studies have investigated in situ the transient effects of biochar addition on soil CO_2 efflux in boreal forests (Li et al. 2018). Palviainen et al. (2018) investigated the long-term effect (after the second summer) of biochar application on carbon and nitrogen fluxes in the same site and found that soil CO_2 effluxes showed no clear response to biochar addition. Only in June, the 1.0 kg m^{-2} biochar (650 °C) treatments had significantly higher CO_2 effluxes compared with the control.

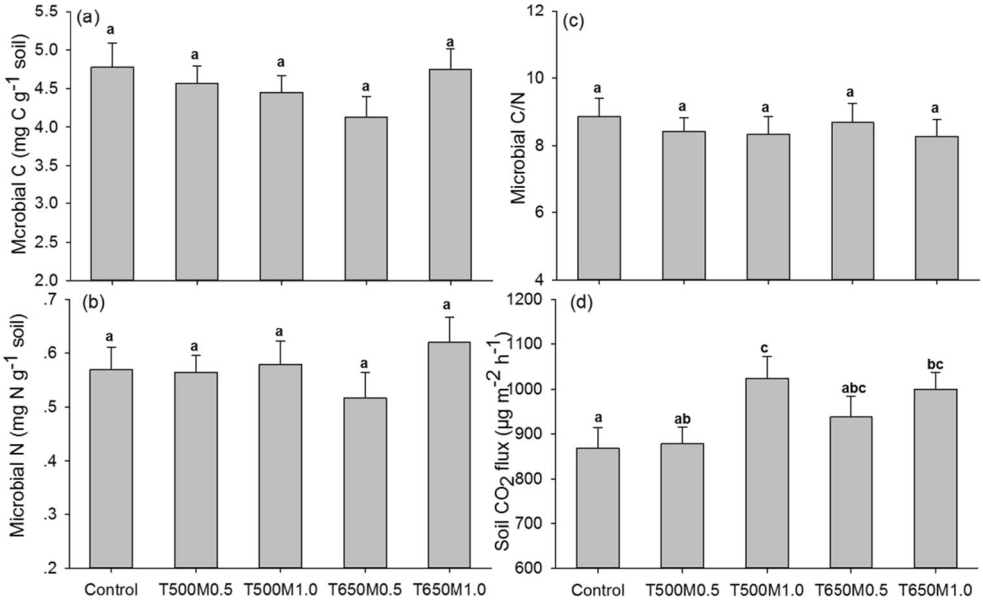

Fig. 2 Mean (± SE) soil microbial biomass C (**a**), mean soil microbial biomass N (**b**), mean soil microbial biomass C-N ratio (**c**) of August and September, and mean soil CO_2 fluxes (**d**) from June to September in different treatments from June to September (6 sample points in each block, 4 blocks for each treatment) (see Fig. 1 for details of treatments). Comparison among treatments was analyzed by linear mixed model with Fisher's least significant difference (LSD) test. Different letters indicate statistically significant differences ($P < 0.05$) between treatments

In our case, soil CO_2 fluxes increased 14.3% ($P < 0.05$) in 1.0 kg m^{-2} biochar treatments and 4.6% ($P > 0.05$) in 0.5 kg m^{-2} biochar treatment relative to the control during the first summer after treatment. This change in soil CO_2 efflux could stem from changes in the physical environment, or in the microbial biomass and activity. Our results suggest that changes in the physical environment dominate the response of soil CO_2 efflux after biochar application. It seems that increases in temperature could be an important cause of the short-term changes in soil respiration. Although our data

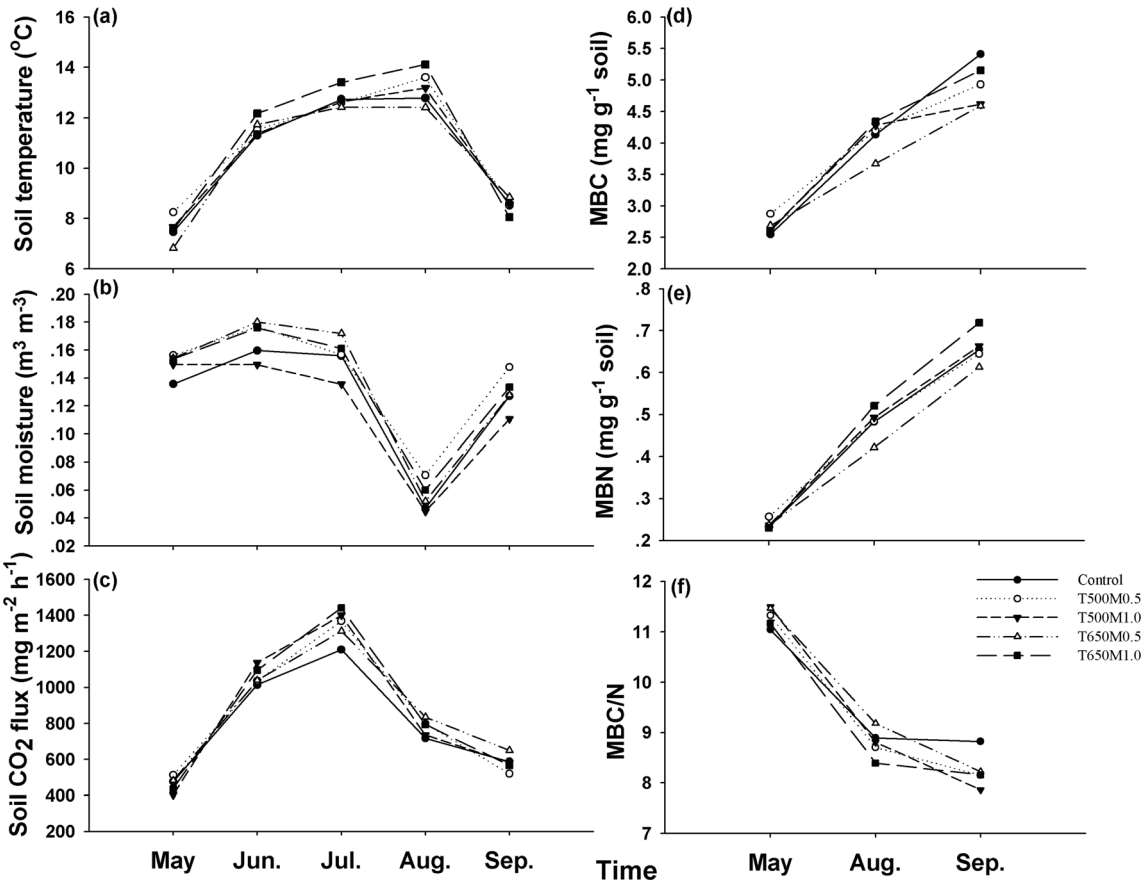

Fig. 3 Monthly dynamics of mean soil temperature at 5-cm depth (**a**), soil moisture (**b**), soil CO_2 fluxes (**c**), soil microbial biomass C (**d**), soil microbial biomass N (**e**), and soil microbial biomass C-N ratio (**f**) with different treatments (see Fig. 1 for details of treatments)

Table 3 Significance of the fixed factors in each linear mixed model. *T* is treatment, *M* is month, *B* is block, *C* is the collar in plot, R_{May} is soil CO_2 efflux in May (prior to treatments) as covariate, *Tem* is soil temperature, *Moi* is soil moisture, and *ln(R)* is natural logarithm of soil CO_2; *a* denotes the coefficients of fixed effects and α denotes the coefficients for random factors; $\mathcal{E}$ is the error term. Equation 5 tests if biochar application increases the soil CO_2 efflux while Eqs. 6–8 test if soil CO_2 efflux differs at a given value of soil moisture and soil temperature

Parameter	Treatment	Soil CO_2 in May	Moisture	Treatment × moisture	Temperature	Treatment × temperature
Equation 5: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + \propto_1 B + \propto_2 C + \varepsilon$	0.048	0.000	-	-	-	-
Equation 6: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Moi} + \propto_1 B + \propto_2 C + \varepsilon$	0.791	0.000	0.001	0.689	-	-
Equation 7: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Tem} + \propto_1 B + \propto_2 C + \varepsilon$	0.816	0.000	-	-	0.000	0.943
Equation 8: $ln(R) = a_1 + a_2T + a_3M + a_4R_{May} + a_5\text{Tem} + a_6\text{Moi} + \propto_1 B + \propto_2 C + \varepsilon$	0.796	0.000	0.000	0.235	0.000	0.921

suggests that soil CO_2 efflux indeed increased after biochar application, the increases at given values of soil moisture and soil temperatures were non-significant (Table 3). This suggests that increases in temperature could be an important cause of soil CO_2 changes. The inconsistent changes of soil moisture among treatments could affect the response of soil CO_2 to temperature but did not change soil CO_2 directly.

Microbial activity in high-latitude soils is strongly limited by temperature, so even small increases in soil temperature may increase soil CO_2 efflux. One main reason for the observed increase in soil temperature in our study could be albedo decreased after biochar application (Vaccari et al. 2011; Genesio et al. 2012), during the whole experimental period, as biochar covered the vegetation on the ground. In contrast, the LAI of our site (Table 1) lack the height to keep the surface albedo unchanged after biochar application. In addition, the amount of biochar affected the increment of soil CO_2, as 1.0 kg m^{-2} treatments had higher density of biochar cover on soil surface and lower albedo than 0.5 kg m^{-2} treatments, which led to higher soil temperature. The increases in soil temperature and CO_2 efflux are probably transient as the biochar particles are incorporated into the soil and covered by vegetation over time (Palviainen et al. 2018). Similarly, Li et al. (2018) reported that initial soil CO_2 increases in biochar experiments are more common than in long-term experiments (exceeding 90 days).

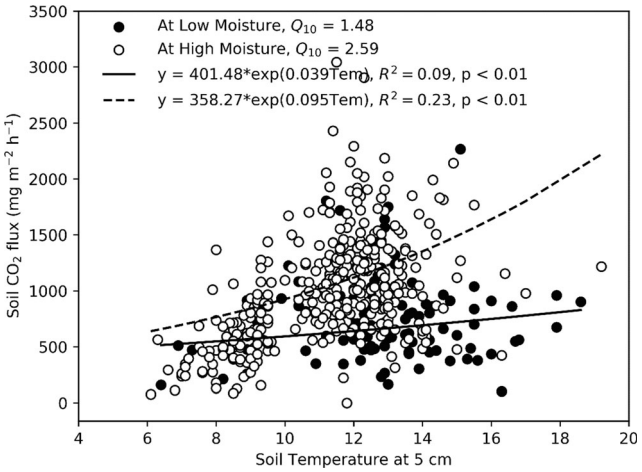

Fig. 4 Regressions of soil temperature and soil CO_2 flux at different conditions in the study sites. Though the differences of soil CO_2 among treatments disappear after correcting by soil moisture and temperature, soil CO_2 flux responds to soil temperature more sensitively at high moisture condition relative to low moisture condition. Q_{10} values for high and low moisture conditions are 2.59 and 1.48, respectively. Each point in the figure corresponds to one measurement of soil CO_2, soil moisture, and soil temperature from one sample point (6 sample points in each plot, 5 treatment plots in each block, 4 blocks in our study from June to September). Hollow dots: high moisture (0.078, 0.412 m^3 m^{-3}); solid dots: low moisture (0.008, 0.076 m^3 m^{-3})

Notably, temperature differences due to biochar amendments (Fig. 1) fall partly within the error range of the sensor (± 0.05% of reading + 0.3 °C). Other possible reasons for increased CO_2 efflux after biochar addition are the decomposition of labile C of biochar, biochar-induced priming effects, or increased plant growth and root biomass (Lehmann et al. 2006; Cross and Sohi 2011). Studies from temperate forests have reported short-term weak positive priming effects or unchanged soil CO_2 (Bruckman et al. 2015; Sackett et al. 2015). Gundale et al. (2016) and Palviainen et al. (2018) applied biochar to boreal forest soil and observed no significant effect on soil CO_2 during two growing seasons. In general, positive priming effects emerge in soils with low C content (Zimmerman et al. 2011). Weak priming effects and moderate changes in CO_2 effluxes in boreal forest soils after biochar addition may occur since boreal forest soils a have high C content (Deluca and Boisvenue 2012). In our case, biochar of 5–10 mm grain size was spread on soil surface; the process of biochar decomposition and mixing with soil take time and do not show the priming effects that other authors had observed.

Biochar application had no significant effect on soil microbial biomass. Other studies also have found negligible changes in microbial biomass after biochar application. After biochar application in a boreal forest, Gundale et al. (2016) observed no large changes in soil respiration, in microbial biomass, or in the

microbial community composition. Noyce et al. (2015) observed that biochar application in a temperate hardwood forest had no significant effect on microbial biomass after treatment. The microbial biomass of the humus layer changed over time in all treatments, probably caused by the seasonal dynamics of environmental factors (Wardle et al. 1998). Our results are consistent with the results of another study of northern forest ecosystems (Gundale et al. 2016), which indicated little or no changes in microbial biomass after biochar addition. The lack of response in microbial biomass supported our conclusion that the main drivers for the increase in soil respiration were changes in the physical environment of soil, especially temperature.

We observed that biochar increased soil pH due to the alkaline nature of biochar along with its high Ca content (Table 1), consistent with other studies (Ventura et al. 2013; Biederman and Harpole 2013; Masto et al. 2013; Zhao et al. 2015; Ahmed and Schoenau 2015). There was a positive relationship between the amount of biochar and soil pH; this was also reported by Kim et al. (2016). Furthermore, electric conductivity increased after the biochar application, which is consistent with the findings of Ventura et al. (2013). The soluble ion concentrations in biochar likely increased the concentration of Ca^{2+} and K^{+} ions in the soil solution and thereby contributed to the increase in soil EC (Kloss et al. 2014). However, our sampling framework did not allow testing for the effect of soil pH and EC on soil CO_2 efflux.

Biochar added to the surface or mixed into the mineral soil can help increase water retention, reduce leaching, or improve bulk density in the soil (Ippolito et al. 2012). Unlike agricultural soils, where biochar can be added and tilled into the soil profile, application of biochar on forest sites is more challenging as trees, stumps, and downed wood hinder movement across a harvest unit (Page-Dumroese et al. 2016). In our study, to avoid soil disturbance and damage to roots, biochar was added to the growing forest and was spread on the soil surface instead of mixing into soil. Little biochar was lost from the area due to wind as the biochar was not powder but particles with 5–10 mm size. The transportation of biochar away with the surface water flow is was unlikely because the terrain is flat, the soil is well-drained coarse sand, and there were no heavy rains during the experiment period. The moss-dominated vegetation did not suffer from biochar addition and remained stable (Palviainen et al. 2018) despite being covered by the biochar during the first growing season.

Previous studies have reported that soil CO_2 efflux increases exponentially with soil temperature (Sheng et al. 2010; Karhu et al. 2011; Liu et al. 2011; Ventura et al. 2013; Song et al. 2013; Pumpanen et al. 2015). This relationship was also found in our study. Fang et al. (2015) proposed that biochar would protect SOM from decomposition by absorbing SOM on its surface leading to lower temperature sensitivity of soil respiration. However, the temperature sensitivity of soil respiration did not change significantly after biochar application. Since our soils were poor in clay minerals that might otherwise protect the biochar from decomposition, we would have expected a marked effect of biochar on the temperature sensitivity of soil respiration. Our data suggests, furthermore, that there are interactions between soil temperature, soil moisture, and soil respiration. Soil CO_2 efflux was more responsive to temperature at high soil moisture contents (Fig. 4). This also explains why CO_2 fluxes decreased sharply in dry August even though the soil temperature was high. The Q_{10} values at high moisture contents were similar to those measured by Pumpanen et al. (2003) at a nearby similar forest site. Altogether, the Q_{10} values measured in the present study were within the range of other studies from boreal forest soils, ranging from 0.98 (Gulledge and Schimel 2000) to 4.75 (Morén and Lindroth 2000). Our data does not suggest that these dependences on humidity and soil temperature would have changed due to the biochar application.

5 Conclusions

Our study on the short-term effects of biochar addition on soil CO_2 efflux, microbial biomass, and soil properties in a boreal Scots pine forest indicated that the initial soil CO_2 efflux responses were dominated by physical effects of biochar on soil temperature. The results showed that the amount of biochar affected soil CO_2 efflux significantly, but pyrolysis temperature of biochar had no effect. Biochar amendment was found to increase soil pH but soil microbial biomass remained unchanged. The increases in soil temperature and CO_2 efflux are probably transient as the biochar particles are incorporated in the soil and covered by vegetation over time. More studies are needed to get the optimum biochar amount for carbon sequestration in boreal forest.

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Data availability The datasets generated during and/or analyzed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.3800097.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 4 The result of the linear mixed model analysis for the effects of treatment on soil temperature, soil moisture, pH, electric conductivity, soil CO_2 fluxes and pairwise comparisons between control and each treatment

Estimates	Mean	Std. Error	df	Pairwise Comparisons	Significant *p*-values
Soil temperature (°C)					
Control	11.365	0.273	4.313		
T500M0.5	11.810	0.273	4.313	Control VS T500M0.5	0.012
T500M1.0	11.763	0.273	4.313	Control VS T500M1.0	0.024
T650M0.5	11.459	0.273	4.313	Control VS T650M0.5	0.590
T650M1.0	12.114	0.273	4.313	Control VS T650M1.0	0.000
Soil moisture (m^2 m^{-2})					
Control	0.122	0.004	472		
T500M0.5	0.136	0.004	472	Control VS T500M0.5	0.029
T500M1.0	0.110	0.004	472	Control VS T500M1.0	0.050
T650M0.5	0.133	0.004	472	Control VS T650M0.5	0.094
T650M1.0	0.133	0.004	472	Control VS T650M1.0	0.103
pH					
Control	3.545	0.052	6.678		
T500M0.5	3.586	0.052	6.678	Control VS T500M0.5	0.274
T500M1.0	3.705	0.052	6.678	Control VS T500M1.0	0.000
T650M0.5	3.633	0.052	6.678	Control VS T650M0.5	0.020
T650M1.0	3.698	0.052	6.678	Control VS T650M1.0	0.000
EC (μs cm^{-1})					
Control	168.917	4.436	110.000		
T500M0.5	183.583	4.436	110.000	Control VS T500M0.5	0.021
T500M1.0	175.000	4.436	110.000	Control VS T500M1.0	0.334
T650M0.5	178.667	4.436	110.000	Control VS T650M0.5	0.123
T650M1.0	173.667	4.436	110.000	Control VS T650M1.0	0.451
Soil CO_2 flux (μg m^{-2} h^{-1})					
Control	868	46.710	110.774		
T500M0.5	878	47.212	110.774	Control VS T500M0.5	0.877
T500M1.0	1024	47.524	111.599	Control VS T500M1.0	0.022
T650M0.5	938	46.756	110.774	Control VS T650M0.5	0.288
T650M1.0	1000	46.800	110.774	Control VS T650M1.0	0.048

Table 5 Estimates of fixed factors in Eq. 8 explaining the relationships between soil CO_2 fluxes and soil temperature and moisture when the treatment factor was considered

Parameter[a]	Estimate	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Intercept (μg m^{-2} h^{-1})	4.439	0.564	0.000	3.333	5.545
[Treatment=T500M0.5]	-0.242	0.461	0.599	-1.148	0.663
[Treatment=T500M1.0]	0.224	0.447	0.617	-0.655	1.103
[Treatment=T650M0.5]	0.091	0.404	0.822	-0.704	0.886
[Treatment=T650M1.0]	-0.190	0.443	0.669	-1.061	0.682
[Treatment=Control]	0[b]	0.000			
Moisture (m^2 m^{-2})	2.625	0.922	0.005	0.813	4.437
[Treatment=T500M0.5] * Moisture	-0.567	1.283	0.659	-3.088	1.954
[Treatment=T500M1.0] * Moisture	0.334	1.395	0.811	-2.407	3.075
[Treatment=T650M0.5] * Moisture	-0.850	1.030	0.409	-2.874	1.173
[Treatment=T650M1.0] * Moisture	1.580	1.347	0.242	-1.068	4.227
[Treatment=Control] * Moisture	0[b]	0.000			
Soil temperature (°C)	0.117	0.025	0.000	0.068	0.167
[Treatment=T500M0.5] * Temperature	0.017	0.034	0.609	-0.049	0.084
[Treatment=T500M1.0] * Temperature	-0.013	0.033	0.702	-0.078	0.052
[Treatment=T650M0.5] * Temperature	0.002	0.031	0.961	-0.060	0.063
[Treatment=T650M1.0] * Temperature	0.002	0.032	0.941	-0.061	0.066
[Treatment=Control] * Temperature	0[b]	0.000			
Covariance Parameters					
May	0.001	0.000	0.000	0.0009	0.0015
Residual	0.208	0.014	0.000	0.182	0.238
Intercept [subject = Area]	0.196	0.000			
Intercept [subject = Area * Collar]	0.0098	0.006	0.128	0.003	0.035

[a] Dependent Variable: Ln(flux)

[b] This parameter is set to zero because it is redundant

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