

ORIGINAL RESEARCH

Biochar increases tree biomass in a managed boreal forest, but does not alter N₂O, CH₄, and CO₂ emissions

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Abstract

Biochar soil amendment may provide the forestry sector with a formidable tool to simultaneously sequester carbon (C) in the soil and aboveground by enhancing plant productivity, yet several key uncertainties remain. Crucially, empirical evidence of long-term effects of biochar management on vegetation and on greenhouse gas emissions in forest ecosystems is scarce. Using a large field experiment in a young managed boreal forest in northern Sweden, we investigated the effects of biochar (applied either on the soil surface or mixed in the soil 8–9 years prior to this study) on supply rates of soil nutrients, on survival and growth of planted *Pinus sylvestris*, on community composition of the understory vegetation, and on forest floor fluxes of N₂O, CH₄, and CO₂. We found that biochar promoted *P. sylvestris* survival only when biochar was applied on the soil surface. Conversely, biochar enhanced *P. sylvestris* growth overall, resulting in a 19% increase in C stored in biomass. Biochar also altered the composition of the understory vegetation, especially when mixed into the soil, and promoted a more resource-conservative community (i.e., with more ericaceous shrubs and less graminoids and forbs). Meanwhile, supply rates of the main soil nutrients were largely unaffected by biochar. Finally, we found that biochar did not alter overall N₂O and CO₂ emissions and CH₄ uptake from the forest floor. Our findings show that biochar amendment increased the net C input to the system, since, besides directly increasing soil C stocks, biochar enhanced biomass growth without increasing soil C losses. Therefore, our study suggests that biochar could potentially be used for emissions abatement in intensively managed boreal forests.

KEYWORDS

carbon sequestration, charcoal, forest management, greenhouse gas emission, *Pinus sylvestris*, plant community composition, soil nutrient supply, tree growth, tree survival, understory vegetation

1 | INTRODUCTION

Amendment of soils with pyrogenic organic matter (charcoal or “biochar”) has been proposed as an effective tool for mitigating climate change (Bruckman & Pumpanen, 2019;

Cowie et al., 2015; Lehmann, 2007). Because biochar is resistant to decomposition, biochar from plant biomass can add to the recalcitrant carbon (C) pool belowground and thus increase the C storage of terrestrial ecosystems (Lehmann et al., 2015; Wang et al., 2016). Furthermore, soil amendment

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with biochar can improve soil fertility, and thus indirectly increase C stocks by enhancing plant growth (Biederman & Harpole, 2013; Palviainen et al., 2020; Thomas & Gale, 2015). However, effects of biochar on soils and plant growth may also alter and increase emissions of the primary greenhouse gases: carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O; Van Zwieten et al., 2015; Wardle et al., 2008). Consideration of how plant biomass and greenhouse gas emissions respond to biochar amendment is therefore key to assessing the potential of biochar as a tool for mitigating climate change.

Biochar-enhanced plant growth has been observed in a variety of ecosystem types as a result of changes in soil chemical, physical, and biological properties (Biederman & Harpole, 2013; Thomas & Gale, 2015). These changes include an increased pH due to a liming effect of biochar (Palviainen et al., 2018), increased availability of nutrients such as N, P, K, Ca, and Mg through enhanced ion exchange capacity (Sackett et al., 2015) and through promotion of microbial activity (Pluchon et al., 2016; Wardle et al., 2008; Xu et al., 2021), and increased water holding capacity (Laird et al., 2010). However, the effect of biochar on plant growth varies widely with environmental context, which includes plant type (Gale et al., 2017; Pluchon et al., 2014), soil and biochar properties (Pluchon et al., 2014), study type (e.g., greenhouse or field experiment, duration; Thomas & Gale, 2015), and environment type (Liu et al., 2013; Thomas & Gale, 2015). Moreover, in natural ecosystems, plant community composition plays a key role on C and nutrient dynamics (Grau-Andrés et al., 2020; Palviainen et al., 2005), yet community response to biochar addition has been explored in only a few studies (Bieser & Thomas, 2019; de Voorde et al., 2014; Gale et al., 2017; Gundale et al., 2016). Given that field studies in natural systems are uncommon (de Voorde et al., 2014; Thomas & Gale, 2015), empirical evidence of the effects of biochar on plant biomass and composition remains limited in many terrestrial ecosystems.

Furthermore, the effects of biochar on greenhouse gas emissions are not well understood, and recent syntheses have highlighted considerable variability in biochar effects across different study systems (Borchard et al., 2019; He et al., 2017; Li et al., 2018). This is in part because biochar-induced changes in production and consumption of greenhouse gases are highly dependent on specific environmental conditions (Xu et al., 2021). For example, biochar-induced changes to the activity and composition of nitrifying and denitrifying microorganisms may impair the production of N₂O and instead promote reduction of N₂O to N₂, thereby reducing N₂O emissions (Borchard et al., 2019; Van Zwieten et al., 2015). Conversely, under some environmental conditions, an increase in soil NH₄⁺ in response to biochar amendment (Gundale et al., 2016) might instead enhance nitrification, and therefore result in higher N₂O emission (Hawthorne

et al., 2017). Regarding methane, higher soil porosity and gas diffusion rates with biochar amendment may promote aerobic methanotrophs, and thus increase the CH₄ sink strength of upland soils (Schnell & King, 1996; Van Zwieten et al., 2015). However, in some ecosystems, biochar-induced changes in soil chemistry may instead inhibit methanotrophic activity, resulting in decreased CH₄ uptake (Hawthorne et al., 2017; Johnson et al., 2017). Despite this context dependency of biochar effects, most research to date has focused on agricultural systems, and field studies on natural ecosystems are still scarce (Bruckman & Pumpanen, 2019). Furthermore, the paucity of data from some major terrestrial biomes, such as forest ecosystems, severely limits our understanding of biochar effects on greenhouse gas emissions.

Boreal forests represent about 30% of the global forested area and exchange large amounts of greenhouse gases with the atmosphere, thus having considerable influence on climate regulation (Gauthier et al., 2015; Pan et al., 2011). Moreover, in some regions, such as Fennoscandia, intensive forest management (mainly for wood production) is carried out in the majority of the forested area (e.g., 79% of the boreal forest area in Sweden; SLU, 2020). Therefore, biochar has the potential to be incorporated into current management strategies across large areas of boreal forest. Very few field studies to date have investigated the effect of biochar on tree biomass in boreal forests (e.g., Bieser & Thomas, 2019; Palviainen et al., 2020; both reporting on effects 3 years after biochar application). Similarly, few field studies in boreal forests have investigated biochar effects on greenhouse gases (e.g., Gundale et al., 2016; Palviainen et al., 2018; Zhu et al., 2020). These studies have focused on short-term (i.e., <2 years) responses of CO₂ fluxes following biochar addition, although Wardle et al. (2008) provide evidence that these effects can persist for 10 years. A more complete quantification of the effect of biochar on tree biomass (i.e., over greater timescales) and of greenhouse gas exchange (i.e., over greater timescales, and including CH₄ and N₂O) is needed to assess the adequacy of using biochar to increase C sequestration in boreal forests.

Here, we use a large-scale field experiment, established in 2011, to examine the effect of biochar soil amendment on biomass of *Pinus sylvestris*, understory community composition, and emissions of greenhouse gases in a young boreal production forest. The experiment consists of a factorial combination of biochar addition and subsurface soil mixing (often referred to as soil scarification in management applications). Survival and growth of *Pinus sylvestris* and abundance of the understory vegetation were measured in 2020, and fluxes of CO₂, CH₄, and N₂O from the forest floor were measured during the growing seasons of 2019 and 2020. We tested the following hypotheses: (1) biochar addition will increase tree biomass by improving soil fertility (i.e., increased availability of N, P, K, Ca, Mg; Gundale et al., 2016); (2) biochar-enhanced soil fertility will also lead to differences

in understory plant composition, by promoting resource-acquisitive understory plant communities; (3a) biochar addition will increase CO₂ emissions, because biochar often increases microbial activity and plant growth due to improved soil conditions (e.g., increased oxygen and nutrient availability; He et al., 2017; Pluchon et al., 2016); (3b) biochar will increase net CH₄ uptake, because biochar addition often increases soil porosity, which in turn can increase CH₄ and oxygen availability to the methanotrophic communities (Schnell & King, 1996; Van Zwieten et al., 2015); (3c) biochar will change N₂O emissions, for example, by potentially altering soil microbial communities that impair N₂O production, or alternatively by increasing NH₄⁺ substrate that enhances N₂O losses associated with nitrification (Borchard et al., 2019; Gundale et al., 2016; Van Zwieten et al., 2015); and (4) for all of the above hypotheses, we expected biochar effects to be greater when combined with soil mixing, because mixing will allow biochar to interact more strongly with soils when it is physically integrated with the mineral soil, compared to when it is placed on the soil surface (Gundale et al., 2016; Makoto et al., 2010). By comprehensively quantifying the response of tree biomass, vegetation composition, and greenhouse gas emissions to biochar soil amendment, we aim to assess the potential of biochar as a management tool for emissions abatement in intensively managed boreal forests.

2 | METHODS

2.1 | Study site and experimental design

The study site covers an approximately 5 ha area located within the Svartberget Experimental Forest, in northern

Sweden (64°13′27″N, 19°48′25″E, 175 m a.s.l.; Figure 1a). The mean annual air temperature is 1.8°C (mean temperature in January is −10.3°C and, in July, 15.3°C), and the mean annual precipitation is 620 mm (data period 1991–2019; Svartberget Research Station, 2020). Snow cover lasts from the end of October to the end of April. The soil is a fine sandy Typic Haplocryod (FAO, Cambic Podzol) formed from silty glacial outwash sediment. The site index is T22–24, which is in line with normal fertility conditions in northern Sweden (Hägglund & Lundmark, 1977). Other studies in the vicinity of the present study have shown that plant growth and soil function in our study system are strongly limited by nutrient availability (Forsmark et al., 2021; From et al., 2016; Maaroufi et al., 2019). Before the start of the experiment, the site had a closed canopy of c. 60-year-old *Pinus sylvestris*, and the understory vegetation was dominated by the ericaceous shrubs *Vaccinium vitis-idaea* and *Calluna vulgaris*.

Full details of the experimental design and implementation are given in Gundale et al. (2016). Briefly, in October 2010, the site was clear-felled (including the removal of tree boles and branches) in line with normal forestry industry practices. In October 2011, four 22.4 × 22.4 m experimental plots were established into each of six blocks (i.e., 24 plots in total). Within each block, plots were randomly assigned to one of four treatments: control, biochar addition only, soil mixing only, and biochar addition followed by soil mixing (Figure 1b). Within blocks, 5 m buffer zones separated each plot, and 10 m buffer zones separated each block. Biochar plots received 500 ± 15 kg (i.e., 10 tonnes ha⁻¹) of locally sourced biochar (see below). In plots assigned to the biochar only treatment, an excavator evenly distributed the biochar on the soil surface using a large excavator bucket. In soil mixing plots, the excavator removed the soil to a depth of

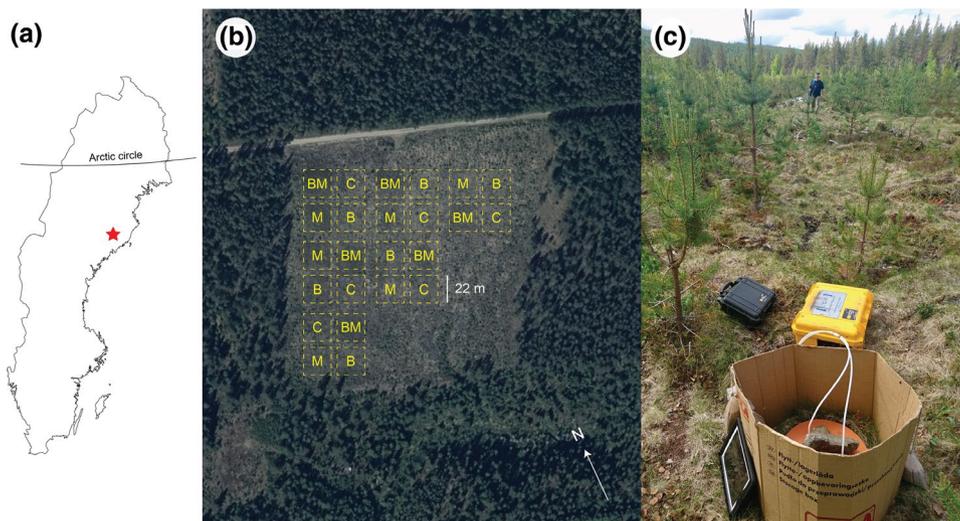


FIGURE 1 (a) Location of the site in northern Sweden (map source: Vemaps). (b) Diagram of the experimental design, with 24 plots belonging to one of four treatments (C = control, B = biochar addition, M = soil mixing, BM = biochar addition + soil mixing) grouped in six blocks. The 0.5 m orthophoto was taken in 2018 (source: Lantmäteriet). (c) Chamber setup for flux measurements with the portable gas analyzer

approximately 30 cm, piled it, and then redistributed the soil evenly over the plot surface. In plots assigned to the mixing and biochar treatment combination, biochar was applied to the plot surface after soil was redistributed, and then was vertically mixed using the excavator bucket. In the spring of 2012, the site was fenced off to eliminate moose browsing, which is a major obstacle for pine regeneration in Northern Sweden, and nursery-grown *P. sylvestris* seedlings (about 1.5 years old and 10 cm tall) were planted at 2 × 2 m spacing using standard commercial procedures.

The biochar was produced by the company Vindelkol AB (Vindeln, Sweden), and is sold commercially as a soil amendment. The biochar was made primarily from *P. sylvestris* feedstock (including a small amount of *Picea abies* and *Betula pendula*) under conditions that the company does not disclose. The biochar had 13.8% ash content (measured following Rajkovich et al., 2012). Biochar pH was 8.04, C concentration was 74%, P concentration was 300 mg kg⁻¹, and concentrations of extractable PO₄⁻-P, NO₃⁻, and NH₄⁺ were 1.26, 0.14, and 1.38 mg kg⁻¹, respectively (Gundale et al., 2016). Previous analyses of the same biochar product reported that particle size distribution was 1–5 mm, effective size was 1.5 mm, specific surface area was 184 m² g⁻¹, bulk density was 187 kg m⁻³, and total porosity was 72%–74% (Dalahmeh et al., 2018).

2.2 | Resin probe nutrients

To assess how changes in soil chemistry may drive the response of vegetation and gas fluxes to the experimental treatments, we measured soil nutrient availability using plant root simulator (PRS) resin probes (Western Ag Innovations). On June 4, 2020, we inserted eight probe pairs into the soil (each pair consisting of a cation probe and an anion probe) at eight random locations evenly spread across each plot. The resin membranes were located 4–10 cm below ground. The probes were collected on July 27, 2020 and immediately cleaned using de-ionized water. On each plot, we pooled four probes into a single sample, that is, we obtained two cation samples and two anion samples per plot. The samples were stored at 4°C until shipped to Western Ag Innovations on August 5, 2020 for ion chromatography analysis of NO₃⁻, NH₄⁺, PO₄⁻, K⁺, SO₄⁻, Ca²⁺, Mg²⁺, Fe, Mn²⁺, Zn²⁺, and Al. We received two data points per plot for each soil ion, which were then averaged to yield a single value for each ion per plot.

2.3 | Vegetation measurements

We measured survival, diameter, and height of all 4680 planted trees (i.e., 195 trees per plot × 24 plots) on September 14–22, 2020, that is, nine growing seasons after the trees were planted. Survival was calculated in each plot as the percentage of living

trees relative to the total number planted. Stem diameter was recorded at 1.3 m height to the nearest tenth of a mm using calipers. Maximum height was measured to the nearest cm using a measuring stick placed next to the stem. We estimated total aboveground biomass of each tree using allometric equations developed for young *P. sylvestris* plantations in Sweden by Claesson et al. (2001). The equations are based on tree height and on stem diameter at 1.3 m height, and include the components stem wood, stem bark, branches, foliage, and dead branches. Dead trees were excluded from diameter, height, and biomass analyses. A conversion factor of 0.50 was used to convert biomass (in dry weight) to C (Thomas & Martin, 2012). Tree biomass was aggregated at the plot level before analysis.

We visually estimated percent cover of each understory species (excluding planted *P. sylvestris*) in 20 randomly positioned 0.5 × 0.5 m quadrats per plot in August 2020. We followed Stenberg and Mossberg (2003) for identification of vascular plant species, and Hallingbäck and Holmåsén (1985) for bryophyte species. Some specimens could not be identified to species level and were grouped at the genus level (e.g., *Cirsium* spp., *Cladina* spp., *Salix* spp.). Lichens other than *Cladina* were grouped into broad foliose or fruticose groups. *Polytrichum commune* could not be confidently differentiated from *Polytrichum formosum* in the field, and both species were grouped together. Taxonomic groups present in less than 5% of plots surveyed (i.e., 24 plots) were excluded from analysis, as their association with experimental treatments was likely unreliable (McCune et al., 2002). Additionally, to infer treatment effects on broad functional groups, we grouped the cover data by the main functional groups, namely, trees, shrubs, graminoids, forbs, pleurocarpous mosses, acrocarpous mosses, and lichens.

2.4 | Gas flux measurement

Ground–atmosphere fluxes of CH₄, CO₂, and N₂O were measured using the static chamber method (Levy et al., 2011). We used a cylindrical opaque PVC chamber with an internal diameter of 29.9 cm and a height of 9.2 cm. On June 2019, we randomly selected five permanent subplot locations in each of the 24 plots. Fluxes of CH₄ and CO₂ were measured on all subplots on each of seven sampling events: June 25 to July 1, 2019; July 29 to August 5, 2019; August 26–28, 2019; June 15–16, 2020; July 28–29, 2020; August 24–25, 2020; and September 15, 2020. We completed 840 measurements in total for each of CH₄ and CO₂, that is, 24 plots × 5 subplots × 7 sampling events. To measure gas fluxes, we sealed the chamber to the ground and recorded CH₄ and CO₂ concentrations in the headspace every 2 s for 4 min using an Ultra-Portable Greenhouse Gas Analyzer (Los Gatos Research). To ensure a good seal during measurement, we inserted the sharp chamber edge a few mm into the ground and kept the chamber pressed to the ground by placing a

weight on top. To further minimize air leakage from the chamber, we placed a wind shield around the chamber to eliminate turbulence around the chamber (Figure 1c). For N₂O, we measured fluxes using the same chamber setup on three subplots per plot (randomly selected) on each of five sampling events: July 2–4, 2019; September 16–19, 2019; June 1–3, 2020; July 31 to August 6, 2020, and September 21–23, 2020. We completed 360 N₂O measurements in total, that is, 24 plots × 3 subplots × 5 sampling events. On each measurement, we took three 30 ml gas samples of the chamber headspace through septa in the chamber using a needle and a 30 ml syringe. Gas samples were taken immediately after sealing the chamber, and 20 and 40 min later. We flushed the syringe before taking the gas sample to mix the chamber headspace. The samples were stored in evacuated 20 ml glass vials and N₂O was analyzed by gas chromatography (Clarus 580 equipped with an ECD detector, PerkinElmer) within a week after sample collection.

Gas flux in each measurement was calculated as described in Levy et al. (2011):

$$F = \frac{dC}{dt_0} \cdot \frac{\rho V}{A},$$

where F is gas flux from the ground (in $\mu\text{mol m}^{-2} \text{s}^{-1}$), dC/dt_0 is the initial rate of change in gas concentration (estimated using linear regression, in ppm s^{-1}), ρ is the air density (in mol m^{-3}), V is the volume of the headspace in the chamber (in m^3), and A is the ground area of the chamber (in m^2). Positive gas fluxes indicate a net efflux from the ground to the atmosphere, and negative fluxes, a net uptake by the ground.

We used the package *FluxCalR* (Zhao, 2019) in R software version 4.0.3 (R Core Team, 2020) to visualize and preprocess the CH₄ and CO₂ data from the portable analyzer. We checked the quality of the N₂O data by comparing the CH₄ and CO₂ results obtained by gas chromatography to the more reliable CH₄ and CO₂ results obtained using the portable analyzer. When CH₄ and CO₂ results obtained by gas chromatography indicated a compromised gas sample (e.g., inconsistent flux patterns or missing data), we excluded the N₂O measurement from analysis. As a result, 54 of 360 measurements of N₂O flux were excluded.

The volume of the headspace in the chamber was calculated as the volume of the chamber minus the volume occupied by the vegetation. To calculate the vegetation volume, we harvested all vegetation from each subplot on September 23, 2020, then vacuum packed the vegetation to remove air, and measured vegetation volume by water displacement. We assumed that the vegetation at the time of harvest was representative of the vegetation over the entire measuring period. Additionally, we measured the temperature and moisture content of the top 10 cm of soil at the same time of gas flux measurement. Soil temperature was measured in each subplot using a temperature probe. To measure soil moisture content,

we took two soil samples (7 cm diameter, 10 cm length) per plot and calculated moisture content gravimetrically by oven-drying the samples at 60°C. The two samples were then averaged to obtain a plot-level measure of soil moisture content.

2.5 | Statistical analysis

All plotting and analyses were completed using the R software, version 4.0.3 (R Core Team, 2020). To account for spatial and temporal dependence among our data, univariate analyses were constructed as linear mixed effects models (package *nlme*; Pinheiro et al., 2020). To test the effect of the experimental treatments on soil nutrient supply, we fitted separate models (i.e., one for each nutrient) which included the binary factors biochar addition and soil mixing, and their interaction, as fixed effects, and block as a random effect. The variables tree survival and tree biomass, which were aggregated and thus also have one value per plot, were tested in exactly the same way. We analyzed tree stem diameter, tree height, and total cover of the understory vegetation by fitting models which included biochar, mixing, and their interaction as fixed effects, and plot nested within block as random effects. Soil temperature and moisture content were analyzed using models with the same fixed effects structure. However, to account for temporal correlation due to repeated measurements across sampling events, these models also included an autocorrelation structure (function “*corAR1*” in *nlme*). To model spatial dependence, we fitted the following as nested random effects: subplot, plot, and block (soil temperature), and plot and block (soil moisture content).

We analyzed CH₄, CO₂, and N₂O fluxes using separate models, with biochar addition and soil mixing, and their interaction, as fixed effects. To account for the effect of environmental variation on gas fluxes (Levy et al., 2012; Zhu et al., 2020), we also included as fixed effects the covariates soil temperature and soil moisture content, and their interaction with biochar addition, soil mixing, and biochar addition × soil mixing. Aboveground vegetation volume was included as an additive fixed effect to account for the effect of understory vegetation on gas fluxes (Levy et al., 2012). Finally, to account for the effect of tree biomass on respiration, we first aggregated the estimated biomass (see above) of the nine planted pines closest to each subplot location, and included this metric as an additive fixed effect on the CO₂ model. Nonindependence among observations due to spatial and temporal correlation was modeled by fitting subplot, plot and block as nested random effects, and sampling event as an autocorrelation structure (function ‘*corAR1*’ in *nlme*). To meet model assumptions of normality and homogeneity of variance, the CO₂ flux data were log-transformed, and, in the CH₄ and N₂O models, a constant variance structure (function “*varIdent*” in *nlme*) across sampling events was applied (Zuur et al., 2009). We used the

package *MuMIn* (Barton, 2020) to calculate R^2 marginal (i.e., the variance explained by the fixed effects) and R^2 conditional (i.e., the variance explained by both fixed and random effects) after Nakagawa et al. (2017). Pairwise comparisons among treatments were computed using the package *emmeans* (Lenth, 2020).

Multivariate analyses of plant community composition were performed using the R package *vegan* (Oksanen et al., 2020). The vegetation cover data grouped by taxonomic group and by functional groups were analyzed separately. We used non-metric multidimensional scaling (NMDS; Clarke, 1993) on the raw data, as implemented in the function “metaMDS,” for visualizing data in ordination diagrams. We selected three-axis NMDS solutions as the best compromise between accuracy and ease of interpretation. The stress values of the NMDS solutions were ≤ 0.15 , indicating a good representation of composition (Clarke, 1993). Treatment variables were fitted onto ordination using the function “envfit” (specifying block as the strata for permutations), and the standard deviation of points was drawn using the function “ordiellipse.” To test differences in composition between experimental treatments, we used permutational multivariate analysis of variance (PERMANOVA), as implemented in the function “adonis2” (Anderson, 2001). PERMANOVA was based on a dissimilarity matrix of the species cover data computed using the “Bray–Curtis” index of dissimilarities, a robust measure for ecological data (Faith et al., 1987). To account for the spatial dependence of our samples, permutations were restricted to within blocks. We used the package *RVAideMemoire* (Hervé, 2020) to carry out pairwise comparisons between treatments groups.

3 | RESULTS

3.1 | Soil nutrient supply, temperature, and moisture content

Biochar addition had no statistically significant effect ($\alpha = 0.05$) on supply of macronutrients NO_3^- , NH_4^+ , PO_4^- , K^+ , and S, but significantly increased Ca^{2+} and Zn^{2+} (Figure 2a–g; Table S1). Soil mixing strongly decreased supply rates of all nutrients (except for S, which was unaltered), but did not mediate the effect of biochar as there were no interactive effects. Soil temperature and soil moisture content were unaffected by biochar addition, but soil mixing increased soil temperature by 0.6°C overall (Figure 2h,i).

3.2 | Vegetation

While biochar had no effect on tree survival overall, the effect of biochar was mediated by soil mixing, because tree survival

significantly increased when biochar was added to the ground surface, but not when biochar was mixed in the soil (Figure 3a; Table S2). Soil mixing strongly increased tree survival (by 70%). Biochar significantly increased *P. sylvestris* stem diameter and height by 17% and 8%, respectively (Figure 3b,c; Table S2). Soil mixing also increased stem diameter (by 57%) and height (by 24%), but there was no interactive effect of soil mixing and biochar. Due to biochar-enhanced tree growth, estimated biomass was increased in biochar plots, by on average 19% (Figure 3d; Table S2). We found a marginally nonsignificant interactive effect of biochar addition and soil mixing ($F_{1,15} = 3.1$, $p = 0.10$) which could be indicative of a greater effect of biochar on tree biomass when applied on the ground surface than when mixed in the soil.

Total cover of the understory vegetation was significantly increased by soil mixing (by 6% cover), but was unaffected by biochar addition or the interaction between biochar and soil mixing (Table S1). In contrast, PERMANOVA showed that biochar significantly affected vegetation community composition, and that soil mixing mediated the effect of biochar, because biochar altered composition when biochar was mixed into the soil, but not when biochar was applied on the ground surface (Table S3). The main effect of soil mixing on composition was greater than the effect of biochar. For example, soil mixing explained 8% of the variation in community composition by taxonomic groups, and biochar, only 1%.

Consistent with the PERMANOVA results, the ordination diagrams show that plant community composition was strongly correlated with soil mixing, and more weakly with biochar (Figure 4a,b). Biochar promoted abundance of the dominant tree (i.e., naturally regenerating *P. sylvestris*) and shrub (i.e., *Calluna vulgaris*), and impaired graminoids (e.g., *Deschampsia flexuosa*, *Calamagrostis lapponica*) and forbs (e.g., *Trientalis europaea*, *Melampyrum pratense*). Soil mixing promoted abundance of trees and some acrocarpous mosses (i.e., *Polytrichum* spp.), and impaired pleurocarpous mosses (mainly *Pleurozium schreberi*), the acrocarpous moss *Dicranum polysetum*, and lichens (dominated by *Cladina* spp.).

3.3 | Gas fluxes

Flux of N_2O from the forest floor was low and averaged zero over the entire measuring period (Figure 5a). Conversely, mean CH_4 flux was $-2.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ (i.e., the forest floor was a net sink of CH_4), and forest floor respiration averaged $3.0 \text{ } \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Figure 5b,c). Flux of N_2O was overall unaffected by biochar addition, but was nearly significantly affected by soil mixing ($F_{1,15} = 3.8$, $p = 0.07$), which increased N_2O uptake from an average efflux of $0.0016 \text{ nmol m}^{-2} \text{ s}^{-1}$ to an uptake of $-0.0053 \text{ nmol m}^{-2} \text{ s}^{-1}$ (Figure 5a; Table S5).

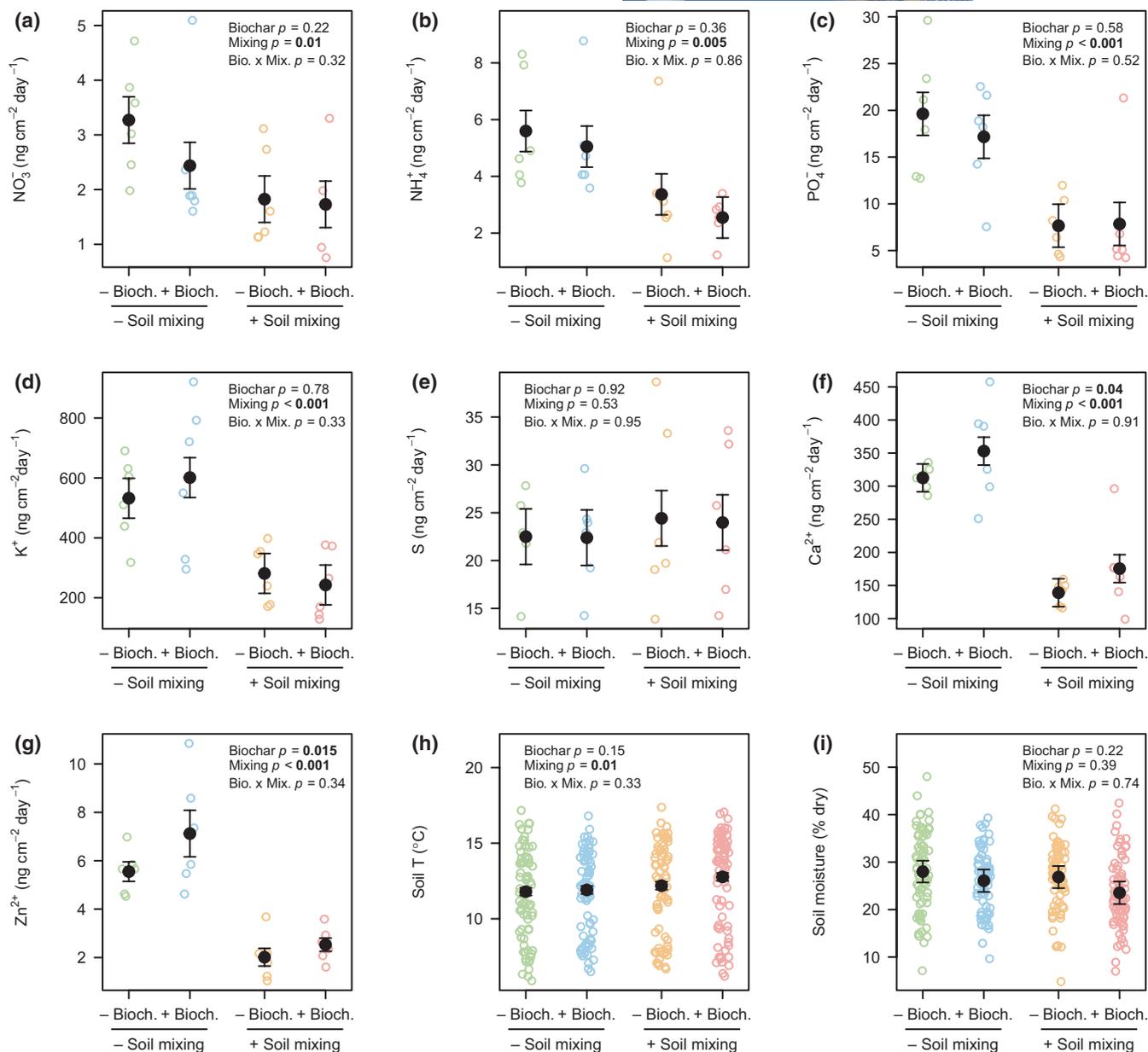


FIGURE 2 Soil nutrient supply rates (a–g), soil temperature (h), and soil moisture content (i) in response to biochar addition and/or soil mixing. For nutrient data, N per treatment was 6 (i.e., 6 blocks \times 1 sampling event), and for soil temperature and moisture content, N per treatment was 72 (i.e., 6 blocks \times 12 sampling events). Open color symbols are individual observations, filled black symbols are mean \pm 1 SE. The statistical results are underpinned by models reported in Table S1. Significant effects at $\alpha = 0.05$ are bolded. Results of supply rates of Mn^{2+} , Mg^{2+} , Fe, and Al are provided in Figure S1 and Table S1

The interaction between biochar and soil mixing did not affect N_2O flux. Flux of CH_4 was unaffected by biochar addition, soil mixing, or their interaction. Conversely, CO_2 efflux was significantly increased by soil mixing (by on average $0.55 \mu\text{mol m}^{-2} \text{s}^{-1}$). Soil mixing also mediated the effect of biochar on CO_2 efflux, through increasing CO_2 efflux when biochar was applied on the soil surface but not when mixed in the soil (Figure 5c; Table S5). Furthermore, we found evidence that soil moisture interacted with biochar ($F_{1,710} = 3.7$, $p = 0.05$) and with soil mixing ($F_{1,710} = 6.3$, $p = 0.01$), because both induced a greater increase in CO_2 efflux in drier

compared to wetter soil (Figure S4). However, biochar had no overall effect on CO_2 efflux.

4 | DISCUSSION

Biochar amendment can increase ecosystem C stocks through adding recalcitrant C belowground and enhancing plant C uptake, and has therefore frequently been proposed for mitigating climate change. However, empirical evidence for the effects of biochar on vegetation and

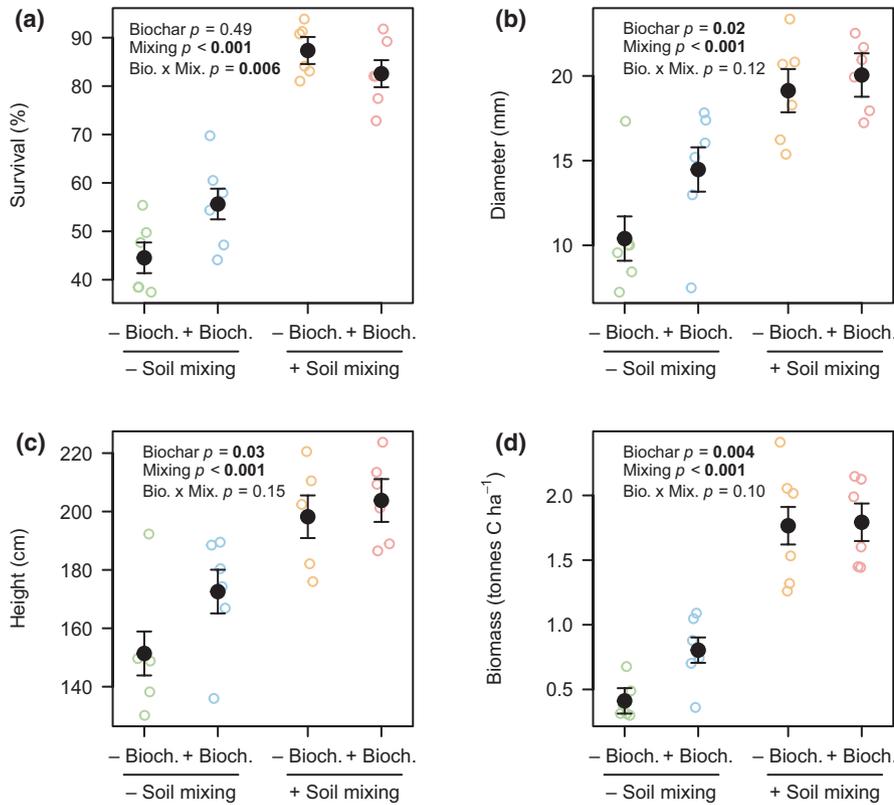


FIGURE 3 Survival (a), stem diameter (b), height (c), and estimated aboveground biomass (d) of planted *Pinus sylvestris* in response to biochar addition and/or soil mixing. Data are aggregated at the plot level (i.e., in each treatment, $N = 6$). Open color symbols are individual observations and filled black symbols are mean ± 1 SE. The statistical results are underpinned by models reported in Table S2. Significant effects at $\alpha = 0.05$ are bolded

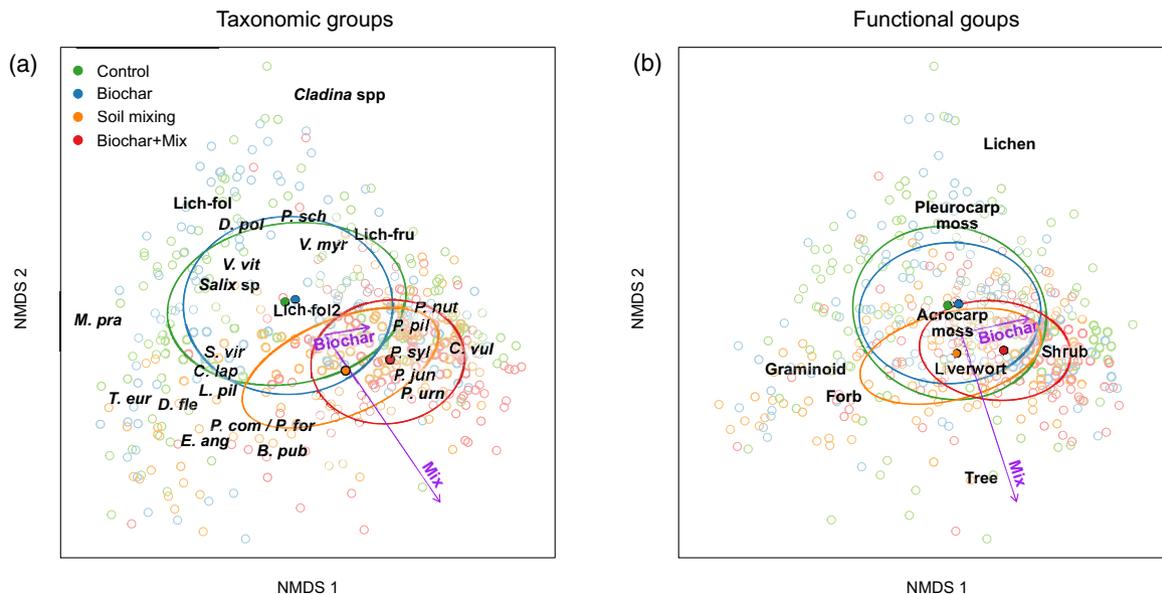


FIGURE 4 Non-metric multidimensional scaling (NMDS) of vegetation cover grouped by (a) taxonomic groups and (b) functional groups. Different colors represent each experimental treatment. Treatment centroids are represented by solid circles, ellipses represent one standard deviation from centroids, and small open circles represent samples (i.e., each 50×50 cm quadrat). Correlation of biochar addition and soil mixing with samples are represented by the direction and length of arrows. Codes in the taxonomic groups NMDS represent: *Betula pubescens*, *Calamagrostis lapponica*, *Calluna vulgaris*, *Cladina* spp., *Deschampsia flexuosa*, *Dicranum polysetum*, *Epilobium angustifolium*, unidentified foliose lichen 1 and 2, unidentified fruticose lichen, *Luzula pilosa*, *Melampyrum pratense*, *Pinus sylvestris*, *Pleurozium schreberi*, *Pogonatum urnigerum*, *Pohlia nutans*, *Polytrichum commune*/Polytrichum formosum, *Polytrichum juniperinum*, *Polytrichum piliferum*, *Salix* sp., *Solidago virgaurea*, *Trientalis europaea*, *Vaccinium myrtillus*, and *Vaccinium vitis-idaea*. Stress values of the NMDS solutions were 0.15 (taxonomic groups) and 0.14 (functional groups). Third NMDS axes are provided in Figure S2. A summary of cover and frequency values of all taxonomic groups in each experimental treatment is provided in Table S4

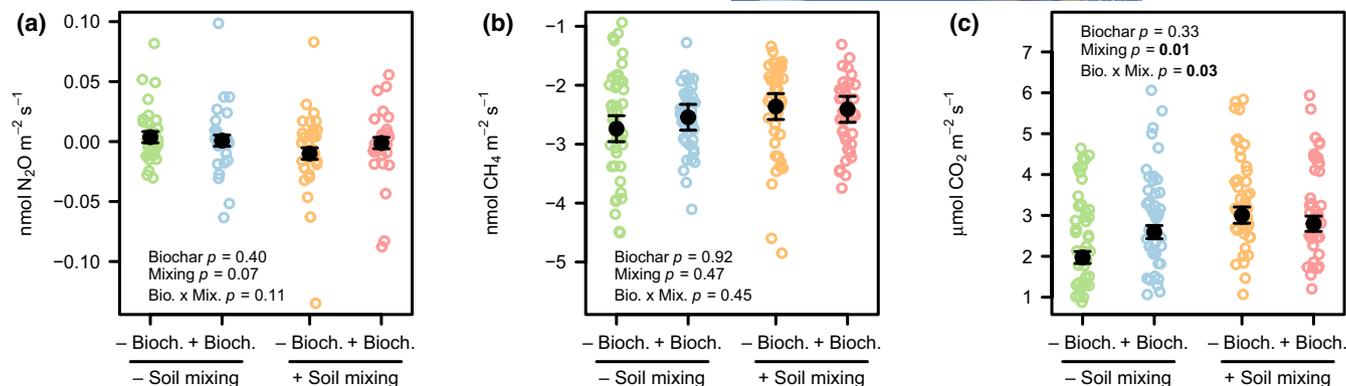


FIGURE 5 Forest floor fluxes of (a) N₂O, (b) CH₄, and (c) CO₂ in response to biochar addition and/or soil mixing. Data were averaged within plot and sampling event. For N₂O, N per treatment is 30 (i.e., 6 blocks \times 5 sampling events), and for CH₄ and CO₂ is 42 (i.e., 6 blocks \times 7 sampling events). Subplot-level data in each treatment and sampling event are presented in Figure S3. Open color symbols are individual observations and filled black symbols are mean \pm 1 SE. The statistical results are underpinned by models reported in Table S5. Significant effects at $\alpha = 0.05$ are bolded

greenhouse gas emissions in forests is scarce and limits our capacity to assess the adequacy of using biochar as a forest management tool. Using a long-term field experiment in a boreal forest, we show that soil amendment with biochar increased survival and growth of planted trees and altered plant community composition but did not have major effects on ground fluxes of CO₂, CH₄, and N₂O.

4.1 | Tree biomass

Our study provides the first empirical evidence of long-term enhancement of tree growth (i.e., over nine growing seasons) by biochar addition in boreal forests. Over the duration of the study, biochar increased C stored in *P. sylvestris* biomass by 19%, in line with our first hypothesis. Compared to previous field studies in boreal forests, our results agree with Palviainen et al. (2020), who also found increased biomass (by 28%) on 13- to 16-year-old *P. sylvestris* stands 3 years after biochar addition, but not with Bieser and Thomas (2019), who found unaltered or decreased growth of *Picea glauca* seedlings 3 years after biochar addition. Our findings on 10-year-old *P. sylvestris* also contrast with research on older conifer trees (>20 years old) in temperate forests showing that biochar had no effect on tree growth (Sarauer et al., 2019), which suggests that younger trees may be more responsive to biochar. The increase in tree biomass in response to biochar was driven by enhanced tree growth, as biochar had no overall effect on tree survival. Over 9 years, biochar enhanced aboveground tree C stores by 0.31 tonnes C ha⁻¹, which, assuming negligible loss of C from biochar (Lehmann et al., 2015; Wardle et al., 2008), is equivalent to 4.2% of the C originally added as biochar (i.e., 10 tonnes biochar ha⁻¹, at 74% C content, as measured by Gundale et al., 2016).

Contrary to our hypothesis that biochar-enhanced plant growth would be associated with higher soil nutrient supply, we found that biochar had no effect on supply rates of the main nutrients N, P, K, and S, although biochar did increase Ca and Zn. It is possible that we did not detect an increased availability of the main soil nutrients in biochar plots because the greater nutrient uptake caused by the higher tree growth in biochar plots may have depleted soil nutrients (Augusto et al., 2002; Jobbágy & Jackson, 2001). For example, our finding that biochar had no effect on NH₄⁺ availability nine growing seasons after the trees were planted contrasts with previous work on our study system showing that biochar enhanced soil NH₄⁺ availability two growing seasons after the trees were planted (Gundale et al., 2016), that is, when tree nutrient uptake was presumably much lower. The depletion of soil nutrients by trees is further supported by our finding that soil mixing induced a strong increase in *P. sylvestris* biomass, and a concomitant decrease in availability of most soil nutrients. It is possible that improved soil physical properties from biochar amendment (e.g., changes to the soil pore structure leading to greater moisture availability; Palviainen et al., 2020) may have also contributed to enhanced growth. Additionally, biochar and soil mixing may have induced a transient enhancement of soil nutrient availability (Gale et al., 2017; Li et al., 2018), which could have had a long-lasting effect on tree growth. As such, current positive effects of biochar on *P. sylvestris* growth might in part be the result of an improved growth environment during early plant development (Gundale et al., 2016; Thomas & Gale, 2015).

Soil mixing tended to dampen the positive effect of biochar on survival (and, possibly, growth) of planted *P. sylvestris*, contrary to our hypothesis that biochar effects would be greater when combined with soil mixing. Given the much greater positive effect of soil mixing on *P. sylvestris* survival and growth compared to the effect of biochar, this suggests

that the effect of biochar was only apparent when limitations to *P. sylvestris* survival had not been alleviated by soil mixing. The positive effect of soil mixing on tree survival and growth could have resulted from improved soil aeration, water retention capacity, and short-term nutrient availability following large mechanical disturbance (Gundale et al., 2016; Piirainen et al., 2007). Furthermore, the effect of soil mixing on improving *P. sylvestris* survival that we report here (i.e., nine growing season after the trees were planted) is very similar to the effect measured two growing seasons after the trees were planted in the same study system (Gundale et al., 2016). This suggests that the effects of soil mixing on increasing tree survival occurred within the first 2 years and indicates that initial effects on tree survival can have lasting impacts on stand development. Overall, our results show that biochar amendment can increase tree biomass in boreal forests through improved tree growth.

4.2 | Understory vegetation

Biochar altered plant community composition, in line with our second hypothesis. However, contrary to our expectation, biochar promoted a more resource-conservative plant community, with higher abundance of resource-conservative ericaceous shrubs (mainly, *C. vulgaris*) and naturally regenerating *P. sylvestris*, and lower abundance of resource-acquisitive graminoids and forbs. Graminoid (e.g., *D. flexuosa*) and forb (e.g., *E. angustifolium*) species are pioneers with high requirements for light and nutrients and may have impaired tree germination and establishment of *C. vulgaris* and *P. sylvestris* through competition (Muukkonen & Mäkipää, 2006; Palviainen et al., 2005). We found no evidence that biochar-induced shifts in resource-acquisition strategies of the understory community were associated with changes in soil nutrient availability, in contrast to observed links between understory trait spectra and soil fertility over longer time periods in boreal forests (Kumordzi et al., 2014; Nilsson & Wardle, 2005). Compared to biochar, soil mixing had a stronger effect on community composition, due to initial extensive mechanical disturbance (Gundale et al., 2016; Palviainen et al., 2005). For example, soil mixing decreased the abundance of the pleurocarpous moss *P. schreberi*, which requires stable environments and dominates late-succession boreal forests, and increased the abundance of pioneer acrocarpous mosses (e.g., *Polytrichum juniperinum*, *Pogonatum urnigerum*) typical of disturbed environments (Jean et al., 2017; Nilsson & Wardle, 2005). Furthermore, soil mixing increased biochar effects, as biochar had a greater effect altering plant community composition when biochar was mixed into the soil, compared to when biochar was applied on top, in line with our predictions. Consistent with this interpretation, previous work has shown that biochar can have a greater

effect on plant growth when biochar is buried in the soil profile, rather than spread on the soil surface, possibly due to enhanced effects in the rhizosphere (Makoto et al., 2010).

Our finding that biochar did not promote soil nutrient supply is contrary to our hypothesis that altered plant community composition in response to biochar would be driven by changes in nutrient supply. The significant shift in plant community composition in response to biochar we observed may have been due to a transient pulse of nutrients (as observed 2 years after the experiment began; Gundale et al., 2016), or to other soil properties that biochar may have enhanced, such as increased porosity and water holding capacity (Laird et al., 2010), or potentially decreased effect of inhibitory secondary metabolites (Wardle et al., 1998). Very few studies have investigated the effect of biochar on plant community composition in boreal forests. Among these, Bieser and Thomas (2019) attributed the change in abundance of several species 3 years after biochar amendment to altered soil nutrient availability and to toxicity effects from biochar. Conversely, Gundale et al. (2016) found in the same study system as our current study that community composition was unaltered 3 years after biochar amendment. This contrasts with our finding that biochar affected composition 9 years after biochar amendment and suggests that the effects of biochar on community composition may take several years to be apparent. Taken together, our results indicate that biochar amendment, especially when mixed into the soil, may promote a more resource-conservative understory community composition.

4.3 | Gas fluxes

Ground fluxes of CO₂, CH₄, and N₂O were generally unaltered by biochar amendment, contrary to our hypotheses that biochar would increase CO₂ emission and CH₄ uptake and alter N₂O emission. Our study provides the first field-based observations of biochar effects on ground CH₄ and N₂O fluxes in boreal forests. For soil CO₂ emissions, our results are contrary to many studies which have reported increased CO₂ efflux in response to biochar (Hawthorne et al., 2017; He et al., 2017; Johnson et al., 2017; Pluchon et al., 2016), including a field study one growing season after biochar was applied in a boreal forest (Zhu et al., 2020). However, our results agree with other field studies in boreal forests, which have reported unaltered CO₂ emissions in response to biochar two growing seasons after biochar amendment (Gundale et al., 2016; Palviainen et al., 2018). Increased CO₂ efflux with biochar has been mainly attributed to enhanced microbial activity following changes in soil physical and chemical properties (Pluchon et al., 2016; Wardle et al., 2008), increased soil temperature and soil moisture (Zhu et al., 2020), and increased plant-associated

respiration (He et al., 2017). While we found that biochar had no major effects on soil nutrient supply, soil temperature, soil moisture content, and cover of the understory vegetation, it is surprising that we did not observe higher soil CO₂ emission in response to enhanced plant-associated respiration in biochar plots, where biomass of planted *P. sylvestris* was increased. Emission of CO₂ increased when biochar was applied on the soil surface, but not when biochar was mixed in the soil, contrary to our hypothesis that biochar-enhanced CO₂ emission would be greater with soil mixing. A possible explanation for this is that enhanced plant-associated respiration increased CO₂ emission only in plots where biochar was applied on the soil surface because, in these plots, biochar seemed to induce a greater increase in *P. sylvestris* biomass. In total, our results indicate that changes in biotic and abiotic properties eight to nine growing seasons after biochar amendment were insufficient to alter overall soil CO₂ efflux.

Our finding that CH₄ was unaltered by biochar amendment is also inconsistent with our third hypothesis that biochar would increase CH₄ uptake. Increased CH₄ uptake has been observed in response to biochar-increased gas diffusion through increased soil porosity, thus enhancing methanotroph activity (Van Zwieten et al., 2015). Our finding that biochar did not alter CH₄ uptake aligns with the few previous biochar field studies in both temperate and tropical forests (Lin et al., 2017; Sackett et al., 2015; Sarauer et al., 2019). Our results suggest that, over the whole measurement period, biochar likely had little effect on gas diffusion, or other mechanisms controlling CH₄ uptake (e.g., inhibition of methanotrophs by NH₄⁺; Van Zwieten et al., 2015). It is possible that biochar did not generally alleviate gas diffusion limitations to methanotrophs in our study system because the coarse-textured Podzols of our study site were already well aerated.

Biochar had no effect on N₂O flux, contrary to our expectation that biochar would alter N₂O flux through changes to the soil microbial composition or soil N availability. Reduced N₂O emission in response to biochar has frequently been observed in agricultural systems and has been proposed to be due to physical or biological immobilization of N compounds, or enhanced microbial reduction of N₂O to N₂ (Borchard et al., 2019; He et al., 2017; Van Zwieten et al., 2015). Much less evidence from natural systems is available. The few field studies carried out in forests to date have reported either reduced or unaltered N₂O emission in response to biochar (Lin et al., 2017; Sarauer et al., 2019). In our study system, the lack of biochar effects on N₂O flux is likely due in part to the extremely low N₂O flux at the site, which probably occurs because nitrogen transformations that lead to N₂O production occur at an extremely low level in boreal soils (Sponseller et al., 2016). Our results address concerns that biochar amendment could increase

N₂O efflux associated with enhanced denitrification (e.g., through greater water holding capacity or greater C substrate in the soil; Van Zwieten et al., 2015) and indicate that biochar amendment does not alter N₂O flux in boreal forests. More generally, given the short-term focus of most studies showing altered CO₂, CH₄, or N₂O fluxes in response to biochar, our long-term observations support the view that biochar effects on greenhouse gas emissions may be short-lived (Borchard et al., 2019).

5 | CONCLUSIONS

Previous research has established that soil amendment with biochar can be an effective management tool to increase belowground C stocks in forests (Bruckman & Pumpanen, 2019; Lehmann, 2007). Here, we show that, in boreal forests, long-term biochar amendment also increases aboveground C stocks through promoting tree growth. Importantly, we also show that ground fluxes of CO₂, CH₄, and N₂O are overall unaltered by long-term biochar amendment, and therefore do not diminish the increased above- and belowground C stocks induced by biochar. Moreover, we observed that biochar promoted a more resource-conservative understory plant community, which could enhance belowground C accumulation (Nilsson & Wardle, 2005). Our study also highlights the need for further research on long-term effects of biochar amendment in forest ecosystems (Borchard et al., 2019). As such, our findings suggest that, while some biochar effects might be transient (e.g., on soil nutrient supply), other effects (e.g., on vegetation composition) are long-lasting and might take several years to become apparent. Overall, our results indicate that biochar amendment in intensively managed boreal forests could potentially be used to mitigate anthropogenic CO₂ emissions (Cowie et al., 2015). Given the importance of boreal forests in the terrestrial C cycle (Pan et al., 2011), large-scale biochar amendment in managed boreal forests could have a substantial effect on greenhouse gas mitigation. However, a greater understanding of the economical and practical considerations associated with biochar management is still required. Further research would benefit from partnering with forest managers to test practical applications of biochar management in case studies.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at <http://doi.org/10.5061/dryad.zpc866t8f>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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