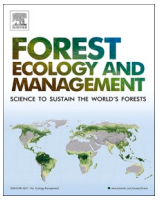
Forest Ecology and Management 534 (2023) 120881

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco



Pyrolysis temperature has greater effects on carbon and nitrogen biogeochemistry than biochar feedstock when applied to a sandy forest soil

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ARTICLE INFO

Keywords:
Greenhouse gases
Soil core incubation
Woody feedstock
Nutrient leaching
Soil respiration
Nitrate

ABSTRACT

Most studies evaluating effects of biochar on soil properties and plant responses have focused on agricultural soils, with limited focus on forested settings. There is increasing interest in applying biochar in forests because of the perceived benefits to soils and productivity, and because biochar could be produced from large quantities of residual woody biomass that is typically unmerchantable due to its size or quality. To test if findings from agricultural soils are applicable to forest soils, we applied biochars created from black ash or balsam fir woody feedstock pyrolyzed at 350 °C or 450 °C to a nutrient-poor forest soil in a laboratory incubation. To assess the influence of biochar on carbon (C) and nitrogen (N) cycling, we measured fluxes of carbon dioxide (CO_2) and nitrous oxide (N_2O), leaching of dissolved inorganic and total N and organic C (DOC), and post-incubation C and N soil chemistry. We found mixed effects, but a generally clear response to pyrolysis temperature. The lower temperature (350 °C) biochar generally increased CO_2 fluxes and had greater N and DOC leaching while the higher temperature (450 °C) biochar appeared to retain N and had little impact on CO_2 fluxes. The black ash feedstock had greater C leaching than balsam fir, but responses were similar across other metrics suggesting that these woody feedstocks produce biochar with similar properties.

1. Introduction

Biochar is a carbon-based material created by heating biomass or waste organic matter at elevated temperatures under low oxygen conditions. There is increasing interest in using biochar as a soil amendment because it can sequester carbon (C) and potentially increase soil fertility and plant productivity (Laird et al., 2017). Biochar has been shown to increase water holding capacity (WHC) (Z. Liu et al., 2017; Omondi et al., 2016) and improve nutrient availability, and thereby enhance crop yield and tree growth (Gogoi et al., 2019; X. Liu et al., 2013; Thomas & Gale, 2015). Pyrolysis is the most common method of producing biochar, generally occurring at temperatures in the range of 400–600 °C (Lehmann & Joseph, 2009). Many studies have demonstrated that the conditions under which biochar is produced (e.g., temperature) affects its properties (Ahmad et al., 2012; Al-Wabel et al., 2013; Gogoi et al., 2019; Hagner et al., 2016), while the feedstock material itself also can influence characteristics of the final biochar product (Ippolito et al., 2020). Feedstocks with high levels of nutrients (e.g., manure) will correspondingly produce biochar with greater nutrient content compared to feedstocks low in initial nutrients (e.g., woody biomass) (Gogoi et al., 2019; Hossain et al., 2020). The interaction between feedstock type and pyrolysis conditions is also important; biochar produced at lower temperatures retains more nutrients, but also volatile compounds which can be toxic to plants (Buss et al., 2015; Deenik et al., 2010). Biochar produced at higher temperatures typically has greater fixed C content, surface area, and cation exchange capacity (CEC), with greater potential for retaining water and adsorbing nutrients (Ippolito et al., 2020). Consideration of how the type of feedstock and pyrolysis conditions will shape the properties of the final biochar product is needed to meet the goals of application (Gogoi et al., 2019; Ippolito et al., 2020).

Biochar has potential to address climate change by increasing C storage and reducing soil greenhouse gas emissions (Gogoi et al., 2019; Thangarajan et al., 2018). Biochar production typically utilizes biomass

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https://doi.org/10.1016/j.foreco.2023.120881
Received 10 November 2022; Received in revised form 14 February 2023; Accepted 16 February 2023
Available online 27 February 2023
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from waste streams, stabilizing the C through pyrolysis, followed by its addition to soil which can potentially sequester C for 1000 years or more (Gogoi et al., 2019; Spokas, 2010). However, a biochar's residence time in the environment is influenced by the stability of the biochar's C and its physical durability, both of which are influenced by pyrolysis temperature and duration, and feedstock (Weber & Quicker, 2018). Carbon content generally increases with pyrolysis temperature, as does the resistance to microbial mineralization, and feedstocks that are more abundant in C result in biochar with greater C contents (Ahmad et al., 2012; Hagner et al., 2016). A key information need is to determine which feedstocks and pyrolysis conditions result in biochar that minimizes the flux of C from soil via gaseous or dissolved exports (Spokas & Reicosky, 2009). Such conditions are not clear; for example, when applied to forest soils, biochar has been shown to have positive, negative, or neutral effects on soil respiration (Gogoi et al., 2019; Mitchell et al., 2015; Zhou et al., 2017). Palviainen et al. (2018) found no effect of biochar produced at two temperatures (500 or 650 °C) and two applications rates (5 and 10 t ha^{-1}) on soil respiration in a boreal forest in the first two years after application. Similarly, Sarauer et al. (2019) found no effect of biochar application at forest sites across western USA at rates up to 25 Mg ha^{-1}. Moreover, the effect of feedstock type and pyrolysis conditions on C leaching (via DOC or particulate transport) is even less clear and understudied. Ideally, the application of biochar should sequester C without promoting the release of C from the soil (above ambient conditions) and without releasing C itself, providing a net C increase to the ecosystem.

Like carbon, nitrogen (N) cycling is influenced by the chemical and physical properties of biochar and how it interacts with specific soil and environmental factors where it is applied (Clough et al., 2013). When applied to forest soils, biochar has been shown to reduce nitrous oxide (N_2O) emissions, potentially due to improved soil aeration and reduced microbial denitrification (Gogoi et al., 2019). Biochar can be a source of N and other nutrients to soil if produced from feedstocks with relatively high initial nutrient contents and can increase nutrient retention in soil because of its CEC (Ippolito et al., 2019; Rasse et al., 2022). However, biochar initially low in nutrients and with a high fixed C content could adsorb nutrients and reduce soil nutrient availability (Hossain et al., 2020; Igalavithana et al., 2015). Some studies reported that net nitrification rates increased up to four-fold after biochar application in forest soils where it was formerly limited (DeLuca et al., 2006). There is still much uncertainty in how feedstock and pyrolysis conditions influence the supply of nutrients for plant growth in both the short- and long-term.

The vast majority of studies evaluating effects of biochar on soil properties and plant responses have focused on agricultural soils, with limited focus on forested settings (Bruckman & Pumpanen, 2019; Li et al., 2018). There is increasing interest in applying biochar in forests because of the perceived benefits to soils and productivity, but also because biochar could be produced from large quantities of residual woody biomass that is typically unmerchantable because of its size or quality (e.g., tree branches following harvest or small diameter trees removed to improve forest health) (Page-Dumroese et al., 2017). However, it is unclear if findings from agricultural soils are applicable to forest soils which are typically more heterogeneous and lower in fertility compared to agricultural soils. These factors make it challenging to predict how a specific forest soil will respond to biochar application.

Biochar undoubtedly interacts with soil nutrients and because it can absorb nutrients from the soil (Hossain et al., 2020; Igalavithana et al., 2015), adding a compost component to 'charge' the biochar may be beneficial (Schulz et al., 2013; Somerville et al., 2020). This practice is even being considered in urban forestry and tree nurseries, but not always with a positive benefit (Simiele et al., 2022; Somerville et al., 2020). For this reason, it is important to understand how a single type of biochar interacts with both low nutrient soils as well as higher nutrient, or fertilized soils.

In this laboratory study, we explored the influence of biochar created from two woody feedstocks at two pyrolysis temperatures on a native, low fertility soil commonly found in forested settings. Our selected feedstocks were black ash (*Fraxinus nigra*), a species continually threatened by emerald ash borer (D'Amato et al., 2018), and balsam fir (*Abies balsamea*), a species that is typically removed from the forest understory but not fully utilized (Lebow et al., 2005). In this sense, the feedstocks we evaluated have high potential to be operationally used for biochar production as part of a forest health management strategy (e.g., Bruckman & Pumpanen, 2019). Our objectives were to assess the impact of biochar created from these two different woody feedstocks at two pyrolysis temperatures (350 °C and 450 °C) on multiple metrics of C and N cycling in a forest soil. Specifically, we measured both gaseous and leaching fluxes of C and N to assess impacts on greenhouse gases and nutrient flow. We also included a compost amendment treatment to identify potential effects masked by the low nutrient content of the forest soil. Our intent was to more fully evaluate potential effects of biochar in the presence of greater nutrient availability to have some inference to higher fertility soils or in situations where fertilizer was applied in tandem with biochar. We hypothesized that the response metrics would vary by pyrolysis temperature, but the black ash and balsam fir woody biochar feedstocks would induce similar soil-nutrient interactions.

2. Methods

2.1. Study area

Soil for this experiment was collected in September 2019 at the University of Minnesota, Cloquet Forestry Center in northern Minnesota, USA (46.5° N, 92.5° W) from a recently harvested (spring 2019) mature red pine (*Pinus resinosa*) stand. The soil is classed as an Omega loamy sand with 0–2% slopes formed from glacial outwash and has low nutrient availability and water holding capacity not uncommon to forest soils (Table 1) (National Cooperative Soil Survey, 2003). The upper 20–30 cm of the soil profile was scraped into a pile with a tractor blade, then sifted through a 1.3 mm (0.5 in.) mesh hardware cloth and sealed in tubs. The tubs were stored outdoors for four months and allowed to freeze, then thawed 19 days prior to the start of the experiment.

2.2. Biochar production

Four biochars were produced from the two woody feedstocks (black ash and balsam fir), subjected to pyrolysis temperatures of 350 °C and 450 °C in a Heyl & Patterson Rotary Calciner Kiln at the Natural Resources Research Institute laboratory in Coleraine, Minnesota. The kiln's shell had a 0.6-m nominal diameter and a 7.5-m overall length with a

Table 1
Soil and compost properties. Total soil carbon, total nitrogen, and extractable elements from the forest soil analyzed prior to the incubation experiment.

Soil Property	Native Soil (n = 10)	Compost[a] (n = 3)
N (w%)[b]	0.043	0.48
C (w%)[b]	0.67	6.3
Ca (mg kg^{-1})[c]	344	
Cu (mg kg^{-1})[c]	0.37	
Fe (mg kg^{-1})[c]	220	
K (mg kg^{-1})[c]	41	
Mg (mg kg^{-1})[c]	85	
Mn (mg kg^{-1})[c]	33	
Na (mg kg^{-1})[c]	29	
P (mg kg^{-1})[c]	51	
Zn (mg kg^{-1})[c]	0.82	

[a] Compost "Guaranteed analysis" from bag: 0.05% TN (0.01% water-soluble, 0.04% insoluble), 0.05% P2O5 (available phosphate), 0.05% K2O (soluble potash).
[b] Analyzed on Leco CHN 628 total combustion analyzer, 950 °C primary furnace, 850 °C afterburner, general method
[c] Analyzed on Thermo Scientific iCap 7000 Series ICP OES, general method

heated length of 4.6 m. The wood feedstocks were chipped to a size of 2 cm and had a moisture content of 10% (w%) at time of production. The temperatures reported (350 °C and 450 °C) correspond to the final product material temperature before exiting the kiln after a residence time of about 15 min. The exterior zones ranged from 0 to 150 °C greater than the reported internal material temperature (data not shown). Properties of the biochar prior to (raw) and following pyrolysis at the two temperatures are shown in Table 2.

2.3. Incubation experiment design and setup

Forty soil cores were filled with mixtures of forest soil, biochar, and compost in a 2x2x2 factorial design with five replicates. The treatments factors were 1) feedstock type (black ask or balsam fir wood biomass), 2) pyrolysis temperature (350 °C or 450 °C), and 3) amendment type (biochar only or biochar + compost). The cores were filled with 2.1 kg field-moist (2 kg dry) forest soil, 100 g dry biochar (5.0% by mass), and compost-amended cores received 161 g moist (100 g dry) compost (5.0% by mass). The compost was a 0.05–0.05–0.05 (N-P-K) manure-derived compost procured from a commercial source (Garick LLC, OH, USA) (Table 1). In addition to the 2x2x2 experiment described above, 10 more cores were filled with forest soil only, or soil plus compost, to serve as non-biochar controls. All soil mixtures were homogenized before filling the cores, then packed by gently tapping the cores vertically on the table.

The cores were built from 10.1-cm (4-inch) schedule 40 PVC pipe with a height of 20 or 30 cm for treatments without and with biochar amendments, respectively (to keep similar headspace between treatment groups). The bottom of each core was fit with a PVC reducing coupling (4-inch to 1.5-inch) and a threaded PVC reducing bushing (1.5-inch to 0.75-inch) with a 63 μm Nitex filter cloth (Science Interactive Group LLC, FL, USA) sealed between the two pieces to limit loss of material. The threaded bushing was fit with a barbed elbow and poly tubing (0.95 mm, 3/8-inch inner diameter) with an in-line ball valve shutoff to act as a drain line (Supplemental Figure S1).

The soil cores were incubated in a walk-in environmental growth chamber (model PGW36, Controlled Environments LTD., Manitoba, Canada) for 49 d following a one-week acclimation period. The chamber was outfitted with sensors (HOBO® U23-002, External Temperature/Relative Humidity (RH) Data Logger, MA, USA) to record temperature and RH at 10-minute intervals. The average temperature and RH for the experiment was 20.0 ± 0.1 °C and 52.4 ± 7.6%, respectively. The filled soil cores were placed in the chamber following a randomized layout and the chamber was kept dark except during sample collection.

Table 2
Biochar properties. Properties of the raw feedstocks and the biochar produced from them by pyrolysis temperature (°C).

	Black Ash			Balsam Fir		
Material Property	Raw	350 °C	450 °C	Raw	350 °C	450 °C
C (w%)	48	76	79	50	68	82
H (w%)	6.1	3.4	3.2	6.1	4.6	3.0
N (w%)	0.2	0.44	0.46	0.2	0.29	0.67
O (w%)	44	16	12	42	25	12
Ash (w%)	1.3	4.7	4.6	0.9	2.1	3.2
VM (w%)	80	32	24	78	46	25
Fixed C (w%)	18	63	72	21	52	72
H:C molar	1.49	0.53	0.49	1.44	0.81	0.44
O:C molar	0.68	0.15	0.12	0.63	0.27	0.11
pH	na	6.2	6.9	na	5.8	6.6
Conductivity (S/m)	na	385	295	na	391	188
Liming (%CCE)	na	3.0	3.0	na	2.0	2.3
CEC (cmol/kg)	na	5.2	3.8	na	3.8	1.3
Water retention (w H20/w BC)	na	3.1	4.2	na	2.7	4.3

2.4. Sampling and analytical procedures

The general sampling protocol was that each week, we applied deionized (DI) water to each core and collected the leachate that drained out, and then measured N_2O and carbon dioxide (CO_2) fluxes the following day. At end of the incubation, subsamples of each core were analyzed for extractable inorganic N and total C and N. Additional details regarding these procedures are described below.

2.4.1. Leachate

The leachate collected each week was analyzed for dissolved organic C (DOC), dissolved total N (DTN), ammonium (NH_4), the sum of nitrite (NO_2) + nitrate (NO_3) (referred to collectively as NO_{2+3}), pH, and oxidation reduction potential (ORP). To collect the leachate, the drain valves were closed, and a fixed volume of DI water was poured onto the soil surface with the goal of recovering approximately 150 ml of drained leachate. Initially, 600 or 700 ml was added to the control (20 cm cores without biochar) and treatment (30 cm cores with biochar) cores, respectively. In effort to maintain a similar volume of leachate collected each week, volumes of DI water were reduced to 300 and 350 ml, then 250 and 300 ml over the course of the incubation as the cores maintained greater moisture and released more drainage. The drainage valves remained closed for an average of 9.0 min before opening and collecting the leachate in 250-ml bottles. Each core released about 100–200 ml (mean 188 ± 40 ml) of leachate.

Oxidation reduction potential of leachate samples was measured within four hours of collection using an Oakton pH 450 m fit with the single-junction, gel-filled, Ag/AgCl ORP electrode and ATC (temp) probe (Oakton® Instruments, IL, USA). The meter was calibrated to 231 mV using Zobell's standard solution before each sample set. The samples were measured for pH within 24 hr of collection using the same Oakton pH 450 m, calibrated each week using a three-point calibration. After the ORP and pH measurements, the samples were refrigerated (~2°C) until being filtered with a 0.45 μm (33 mm housing) nylon syringe filter (Fisherbrand™). All leachate samples were filtered within 16 days of collection (40% within three days and 60% within eight days). The filtrate was split into one 35-ml sample which was acidified with HCl for DOC and DTN analysis, and one 15-ml sample for inorganic N analysis. The filtered samples were refrigerated until analyzed using a colorimetric flow-through analyzer (Lachat, Loveland, CO) for NH_4 and $NO_2 + NO_3$ (Mulvaney, 1996) or sent to the Research Analytical Laboratory on the University of Minnesota Twin Cities campus for DOC and DTN analysis by combustion on a TOC Select Analyzer (Elementar Americas Inc., NY, USA) equipped with an IR Detector (for C) and an Electrical Conductivity Cell (for N). The NH_4 and NO_{2+3} samples were in cold storage for nine to thirty-five days (mean 20 days) before analysis except for 75 samples (14%) that were frozen and analyzed 162 days after collection. The acidified DOC and DTN samples were in cold storage for 26 to 42 days (mean 33 days) before analysis except for 37% of samples that were frozen and analyzed 99 to 113 days after collection.

2.4.2. Gas fluxes

Gas fluxes were quantified weekly using methods previously applied in similar soil core incubation experiments (Berryman et al., 2009; Toczydlowski et al., 2020). To collect gas samples, a tightly fitting PVC cap was placed over each core and 12-ml syringe samples were drawn through a septum 20 and 40 min after cap placement. The PVC caps were vented during placement to avoid pressure buildup in the core headspace. The soil cores were sampled consecutively inside the sealed environmental chamber (with ventilation) over approximately five hours. "Time zero," ambient air reference samples were collected in the chamber at the beginning, middle, and end of the sampling period. Gas samples were immediately transferred to 9-ml glass vials sealed with butyl rubber septa and analyzed within 48 h using two gas chromatographs (Agilent/Hewlett-Packard, Palo Alto, CA), one equipped with a thermal conductivity detector to measure CO_2 and an electron capture

detector for N_2O, and the other equipped with a flame ionization detector to measure methane (CH_4), both connected to a headspace autosampler (Teledyne Tekmar, Mason, OH). A 14-port valve (Valco Instruments, Houston, TX) split each gas sample into three separate sample loops. Only two sets of gas samples were analyzed for CH_4, one near the beginning (day 9) and end (day 49) of the experiment.

Gas fluxes were calculated using:

$$F = \frac{V}{A}\left(\frac{dC}{dt}\right)$$

where $\frac{dC}{dt}$ is the linear rate of change in gas concentration (g m^{-3}), V is the headspace volume for each soil core (0.0009 m^3 on average), and A is the enclosed soil surface area (0.0080 m^2).

2.4.3. Post-incubation analysis

Upon the conclusion of the experiment, the material from each core was homogenized and subsampled. A portion of the subsamples were air-dried and archived for subsequent total C and N analysis. Within 48 hr of collection, separate 15.0-g (±0.1 g) (undried) subsamples were extracted in 45.0 ml of 2 M KCl by shaking for one hour, resting for one hour, then filtering using 0.45 μm nylon syringe filters. The filtered samples were frozen and stored until analysis for NH_4 and NO_{2+3} using a colorimetric flow-through analyzer (Lachat, Loveland, CO) (Mulvaney, 1996; Venterea et al., 2015). In early 2022, the archived soil samples were analyzed for total C and N by combustion analysis using an elemental analyzer (vario MACRO cube, Elementar, USA).

2.4.4. Statistical analysis

All statistical analyses were conducted using R software (R Core Team, 2017). Since the control cores, by definition, did not have an associated feedstock or pyrolysis temperature, we took two approaches to the analysis of treatment effects to utilize data from the controls. First, we analyzed the data in a 2x2x2 (feedstock × pyrolysis × compost) structure excluding the control groups. This allowed us to test feedstock, pyrolysis temperature, and compost all as main effects with their interactions, but without comparisons against the control. Second, we analyzed the data in a 5x2 structure (biochar × compost) with a 5-level biochar factor (no biochar, and four biochar treatments: 350 °C and 450 °C balsam fir, and 350 °C and 450 °C black ash). This allowed us to test biochar treatments against the control groups. Because our main objective was to test the effect of feedstock type and pyrolysis temperature, most figures show the first method of analysis with the control group(s) as a reference. Additional figures including comparisons to controls are included in the supplemental materials.

The leachate metrics (DOC and N species) were analyzed by concentration, then by mass (concentration times volume of leachate collected each sample period), then finally the cumulative mass was calculated for the entire experiment (sum of each week's mass). For simplicity, we presented only analysis of the cumulative mass data. Cumulative gas fluxes were calculated using trapezoidal integration and the totals were analyzed similarly to the cumulative mass for the leachate metrics. Treatment effects and their interactions on leachate and gas flux responses were analyzed using ANOVA. When significant effects were found, differences among treatments were tested using least-squared means (LSM) analyses with the 'lsmeans' R package using a Bonferroni adjustment to control the Type 2 error rate (Lenth, 2016). Data from the post-incubation measurements (inorganic N, total C, and total N measurements) were analyzed similarly. The inorganic N species (NH_4, and NO_{2+3}) and DTN were first analyzed individually, then dissolved organic nitrogen (DON) was calculated by subtracting NH_4 and NO_{2+3} from DTN and analyzed separately.

The 'powerTransform' tool in the 'car' R package was used to identify the best transformation when needed to stabilize the variance (Fox & Weisberg, 2011). Transformations were only necessary for the post-incubation extractable N data, and therefore un-transformed data are grouped and reported appropriately to show significant model interactions. An alpha level of 0.05 was used in all statistical tests to determine significant effects.

3. Results

3.1. Gas fluxes

The cumulative CO_2 fluxes (integration of all weeks) encompass variations from week to week, providing a comprehensive evaluation of the effect of biochar over the entire study period. In the cumulative flux model (2x2x2 analysis), there was significant interaction between the feedstock and compost factors ($p = 0.02$), where the addition of compost increased CO_2 fluxes by 48% in the balsam fir biochar, but not in the black ash biochar. The main effect of pyrolysis was also significant ($p < 0.001$) which manifested as greater CO_2 flux in the 350 °C biochar treatment than the 450 °C biochar treatment and control (cumulative CO_2 flux of 52.5 ± 2.1 g C m^{-2}, 34.6 ± 2.1 g C m^{-2}, and 43.7 ± 4.8 g C m^{-2} in the 350 °C biochar, 450 °C biochar, and control, respectively). In testing against the control (5x2 analysis), the 350 °C black ash biochar produced significantly more CO_2 than the control (68% increase, Table 3, Fig. 1); a similar non-significant response was observed for the 350 °C balsam fir biochar. In contrast, the 450 °C biochar (both feedstocks) had very similar or reduced amounts of CO_2 compared to the control (Fig. 1).

There was a main effect of compost on cumulative N_2O fluxes (Table 3), but fluxes were generally very low (<0.2 mg N m^{-2} d^{-1}, data not shown). The compost amendment decreased N_2O fluxes by 27% relative to no-compost treatments, 2.56 ± 0.28 mg N m^{-2} and 1.87 ± 0.27 mg N m^{-2} respectively. There was no effect of pyrolysis ($p = 0.06$) or feedstock treatments ($p = 0.48$), and no significant interactions among the factors (Table 3). The results were similar when using the 5x2 analysis, testing with the control, and no significant treatment effects were observed (Table 3).

Measured CH_4 fluxes on days 9 and 49 were slightly negative and not different from zero, generally between 1 and −2 μg C m^{-2} d^{-1} (data not shown).

3.2. Leachate

3.2.1. Dissolved organic carbon

In the 2x2x2 analysis of accumulated DOC mass in leachate over the course of the experiment, there was a significant interaction between feedstock and pyrolysis treatments ($p < 0.001$), (Table 4). The 350 °C biochar leached significantly more DOC than the 450 °C biochar for both feedstocks, but the magnitude of the flux was more pronounced in the black ash biochar (Fig. 2). The black ash biochar had approximately 90%

Table 3
ANOVA results for gas flux models. Summary of F-statistic probabilities and degrees of freedom for three-way ANOVA results for cumulative gas flux models using the 2x2x2 analysis approach (no control) and the two-way ANOVA results for the 5x2 (with control) analysis approach.

		CO_2	N_2O
Model Term	Num DF	p-value	p-value
2x2x2 analysis			
Feedstock	1	0.1110	0.4789
Pyrolysis	1	<0.0001	0.0602
Compost	1	<0.0001	0.0458
Feedstock:Pyrolysis	1	0.7771	0.2839
Feedstock:Compost	1	0.0201	0.5035
Pyrolysis:Compost	1	0.1268	0.4548
Feedstock:Pyrolysis:Compost	32	0.7382	0.3279
5x2 analysis			
Biochar	4	<0.0001	0.2396
Compost	1	<0.0001	0.0744
Biochar:Compost	4	0.0187	0.5724

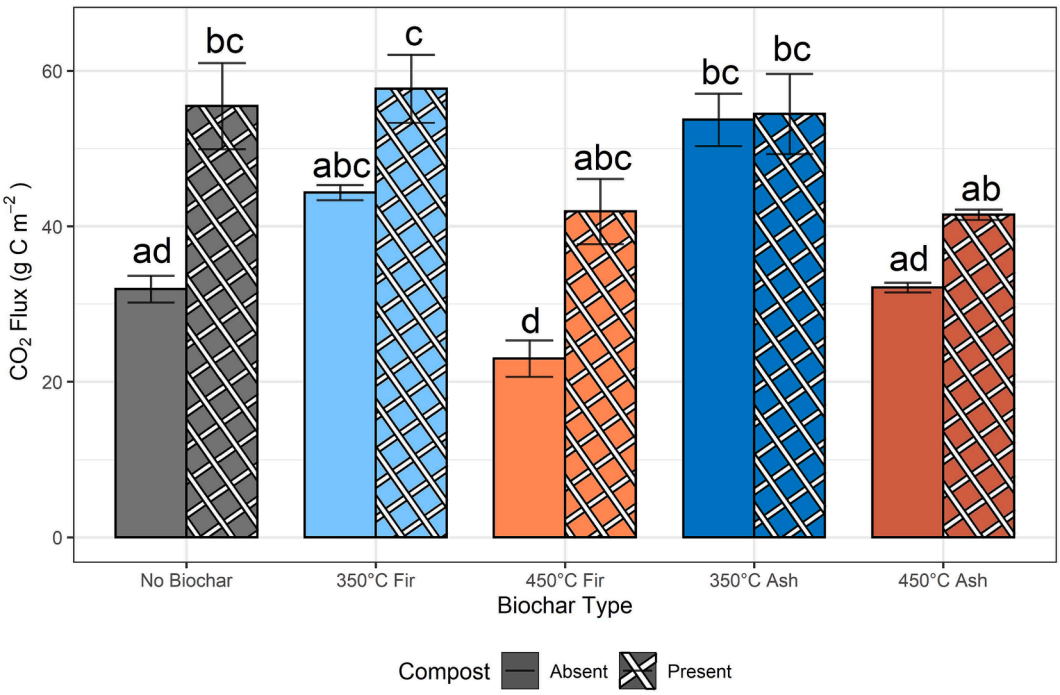

Fig. 1. Cumulative CO_2 fluxes. Total cumulative CO_2 gas fluxes (g C m^{-2}), summed over 49 days, shown using the 5x2 (biochar × compost) analysis. Error bars show standard error. Letters indicate significant differences between treatments ($p < 0.05$). Crosshatched and solid bars represent treatments with and without compost respectively.

Table 4
ANOVA results for leachate models. Summary of F-statistic probabilities and degrees of freedom for three-way ANOVA results for leachate models (total accumulated mass) using the 2x2x2 analysis approach (no control) and the two-way ANOVA results for the 5x2 (with control) analysis approach.

		DOC	DTN	DON	NH_4	NO_{2+3}
Model Term	Num DF	p-value	p-value	p-value	p-value	p-value
2x2x2 analysis						
Feedstock	1	<0.0001	<0.0001	<0.0001	0.3059	0.3683
Pyrolysis	1	<0.0001	<0.0001	<0.0001	0.0081	0.8245
Compost	1	<0.0001	<0.0001	<0.0001	<0.0001	0.0007
Feedstock: Pyrolysis	1	<0.0001	0.0002	0.0239	0.0003	0.6924
Feedstock: Compost	1	0.9313	0.0957	0.3226	0.6966	0.0335
Pyrolysis: Compost	1	0.6022	0.0109	0.1685	0.0182	0.5077
Feedstock: Pyrolysis: Compost	32	0.5988	0.0836	0.1268	0.8290	0.6119
5x2 analysis						
Biochar	4	<0.0001	<0.0001	<0.0001	0.0008	0.3778
Compost	1	<0.0001	<0.0001	<0.0001	<0.0001	0.0034
Biochar: Compost	4	0.7116	<0.0001	0.2762	0.0008	0.1304

more DOC in the 350 °C treatments and approximately 50% more at 450 °C, relative to balsam fir at those same temperatures. For both feedstocks, the 350 °C biochar had greater DOC leaching than the control, but there was no difference between the control and the 450 °C treatments (Supplemental Figure S2). There was also a main effect of compost ($p < 0.001$) where treatment groups amended with compost leached greater amounts of DOC than those without compost, 90.9 ± 8 mg vs 59.2 ± 8.1 mg, respectively.

3.2.2. *Dissolved nitrogen*

When assessing the cumulative mass of N leached over the incubation period, there was significant interaction between feedstock and pyrolysis on DTN, DON, and NH_4 (Table 4). Patterns of response were the same for all N forms; for both black ash and balsam fir feedstocks, the 350 °C biochar leached significantly more DTN, DON, and NH_4 (black ash only) than the 450 °C biochar, ranging from 38% to 140% depending on feedstock and N form (Fig. 3). A second common response pattern was that more N was leached from the black ash biochar produced at 350 °C compared to the balsam fir biochar produced at 350 °C. There was also significant interaction between the pyrolysis and compost factors for DTN and NH_4 (Table 4). In those instances, the interaction manifested as increased N when compost was added for each pyrolysis temperature (Fig. 4). However, for both compost treatments, DTN and NH_4 were higher in the 350 °C pyrolysis treatment relative to the 450 °C treatment (by 54% and 44% for the treatments with and without compost, respectively). Without compost, the 350 °C black ash treatment had significantly greater DTN than the control group and the other treatments in the 5x2 analysis (Supplemental Figure S3, Table 4). With the addition of compost, DTN in the 350 °C black ash treatment was still significantly greater than the control, and 450 °C was significantly reduced for both feedstocks. Similarly, for NH_4, the 450 °C treatments with compost were significantly lower than the corresponding control, as was the 350 °C balsam fir feedstock (Supplemental Figure S3).

The only significant treatment effects in the analysis of cumulative NO_{2+3} mass were the main effect of compost ($p = 0.001$) and the interaction between feedstock and compost ($p = 0.03$) (Table 4). In the balsam fir feedstock, the compost amendment increased NO_{2+3} leaching by 50% (data not shown). Compost did not have a significant effect on NO_{2+3} leaching in the black ash feedstock. In the presence of compost, the addition of biochar appeared to decrease NO_{2+3} leaching compared to the control, although the difference was not significant in the 5x2 analysis with the control (Table 4, Supplemental Figure S3).

Based on the difference in magnitudes between DTN and NH_4 and NO_{2+3}, there is a greater amount of organic nitrogen than mineral nitrogen in the leachate. Moreover, because of the analytical methods, the

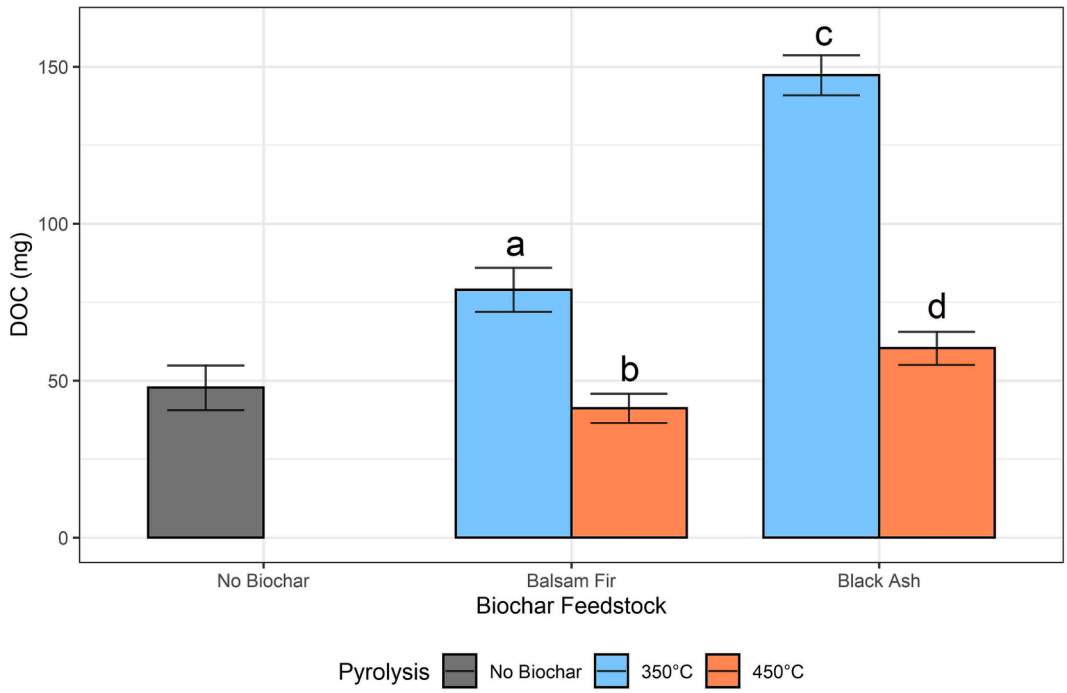

Fig. 2. Accumulated DOC. Accumulated DOC (total mass from each collection extrapolated over volume and summed over 43 days) in the leachate, summarized to show the feedstock-pyrolysis interaction. Error bars show standard error. Letters indicate significant differences between treatments ($p < 0.05$).

detection limit of DTN was 1.0 mg L^{-1}, potentially deflating the magnitude of DTN in this analysis.

3.2.3. Other metrics

In addition to nitrogen and carbon, we measured pH and ORP on the leachate samples. The pH increased slightly during the incubation from a mean of 6.07 (±0.03) in the first week to 6.64 (±0.03) in the third week then plateaued (Supplemental Figure S4). Across all treatments, the experimental mean pH was 6.59 (±0.02). In analyzing the treatment means, feedstock was not a significant factor ($p = 0.12$), but pyrolysis ($p < 0.001$) and compost ($p = 0.008$) were (Table 5). Leachate pH increased with biochar application and pyrolysis temperature with mean pH of 6.52 (±0.03), 6.54 (±0.02), and 6.67 (±0.03) for the control, 350 °C, and 450 °C biochar respectively. The compost amendment increased mean pH significantly ($p = 0.008$) from 6.56 (±0.02) to 6.62 (±0.02). In the 5x2 analysis this interaction of biochar and compost was significant ($p = 0.04$) (Table 5). For both feedstocks at 450 °C, the treatments with compost had significantly higher pH than the control with compost.

Oxidation reduction potential of the leachate generally decreased over the incubation. In the first week the average ORP was 268 ± 3.5 mV, then it gradually decreased to 237 ± 1.9 mV in the fifth week and stabilized (Supplemental Figure S5). The overall mean ORP was 247 ± 1.2 mV. When comparing treatment means, the feedstock-pyrolysis ($p = 0.03$) and pyrolysis-compost ($p = 0.006$) interactions were significant (Table 5). In the feedstock-pyrolysis interaction there was no difference among the 450 °C biochars, but at 350 °C the balsam fir biochar had significantly higher ORP compared to the black ash biochar (Table 6). In the pyrolysis-compost interaction, the compost amendment significantly decreased ORP for both 350 °C and 450 °C pyrolysis temperatures. When testing with the control using the 5x2 analysis approach, biochar did not have a significant effect on ORP (Table 5).

3.3. Post-incubation metrics

In the soil extraction following the incubation, there were similar concentrations and response patterns for NH_4 and NO_{2+3} across treatments, so we simplified the analysis to focus on total extractable N (TEN). There was a significant interaction ($p < 0.001$) between pyrolysis and compost on TEN (Table 7). In the absence of compost, the 350 °C and 450 °C pyrolysis treatments had similar amounts of TEN and both appear lower than the control (Fig. 5). The addition of compost significantly increased the amount of TEN at both pyrolysis temperatures relative to groups without compost, but the increase was smaller in the 450 °C pyrolysis treatment compared to the 350 °C treatment. Compost increased total extractable N by 118%, 188%, and 47% in the control (native soil), 350 °C, and 450 °C biochar respectively (Fig. 5). The addition of biochar appeared to decrease the amount of TEN, especially at the 450 °C pyrolysis temperature, however, in the 5x2 analysis testing against the control none of the treatment groups differed significantly from the control (Table 7).

There was a significant interaction among feedstock, pyrolysis, and compost treatments on total soil N (TN) at the end of the experiment ($p = 0.002$) (Table 7). While the interactions are complex, of the treatment groups without compost, the 350 °C black ash biochar had significantly greater TN than the other groups (Supplemental Figure S6). Among the treatment groups with compost, the 350 °C biochar generally had more TN than the 450 °C biochar, but this was only significant for the balsam fir feedstock. In all cases, the compost treatment groups had significantly more TN than their counterparts without compost. The amount of TN is similar in the biochar treatments and the control groups, except that the 350 °C black ash without compost has significantly more TN than the control without compost (Supplemental Figure S7, Table 7). The post-incubation values of TN in treatment groups without compost also appear to be similar to the pre-incubation soil except for the elevated 350 °C black ash biochar, but we were not able to test this statistically (Supplemental Figure S7). A similar analysis of post-incubation soil C showed no significant main effects of feedstock ($p = 0.08$), pyrolysis ($p = 0.14$), or compost ($p = 0.09$) (Table 7). The feedstock-pyrolysis interaction was significant ($p = 0.04$). However, no significant differences were noted in the pairwise comparison. The addition of biochar did increase total soil C by about 350% compared to the control (Supplemental Figure S8).

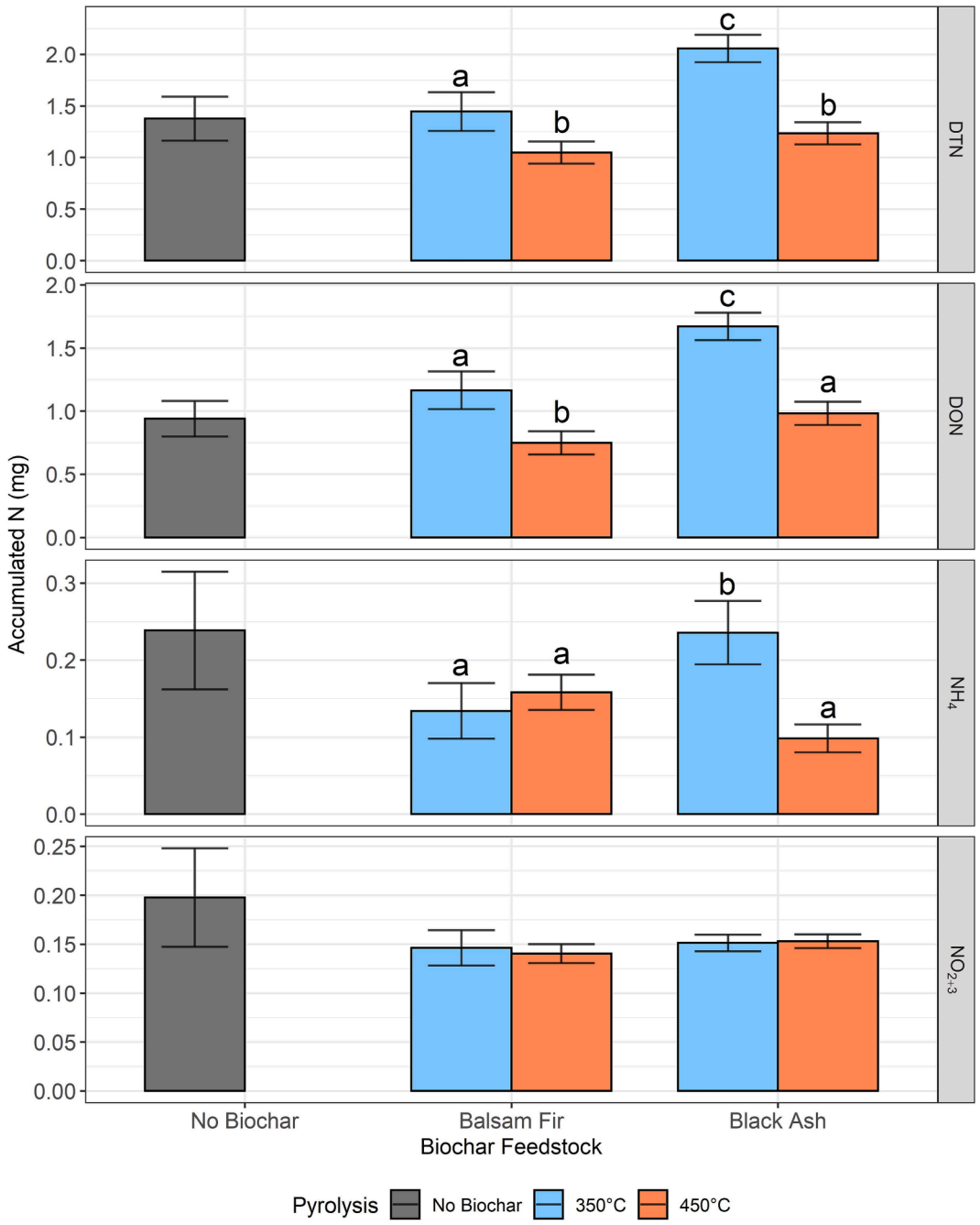

Fig. 3. Dissolved N feedstock:pyrolysis. Accumulated dissolved N (total mass from each collection extrapolated over volume and summed over 43 days) in the leachate, summarized to show the feedstock-pyrolysis interaction. Error bars show standard error. Letters indicate significant differences between treatments ($p < 0.05$).

4. Discussion

4.1. Carbon

In this experiment we assessed C cycling through gaseous fluxes, leachate flushes, and total soil C. Prior to the addition of biochar or compost, the native forest soil had<1% total C (0.67%). As expected, the addition of biochar increased total soil C to greater than 3% (3.19–3.52%). However, there were distinct differences in C fluxes between the two pyrolysis treatments, with the 350 °C treatment having significantly greater loss of C via microbial respiration and leaching of DOC. These findings may be due to the lower pyrolysis temperatures creating a biochar with reduced aromaticity (Greco et al., 2021; Zhang et al., 2021) and higher amounts of water extractable compounds (DOM/DOC) than biochars produced at higher temperatures (Godlewska et al., 2021). There was also a difference in feedstock where 350 °C black ash had both higher CO_2 fluxes (without compost) and greater DOC leaching than the 350 °C balsam fir. Note that the black ash biochar did have a slightly greater C content than the balsam fir at 350 °C (Table 2). Other studies that evaluated effects of biochar application on forest soil respiration have found mixed results with increased (Hawthorne et al., 2017; Mitchell et al., 2015), decreased (Sun et al., 2014) and neutral effects (Gogoi et al., 2019; Malghani et al., 2013; Sackett et al., 2015) on CO_2 flux in response to amending forest soils with biochar. Hawthorne et al. (2017) saw increased CO_2 fluxes with biochar made from Douglas fir pyrolyzed at 420 °C applied at 1% and

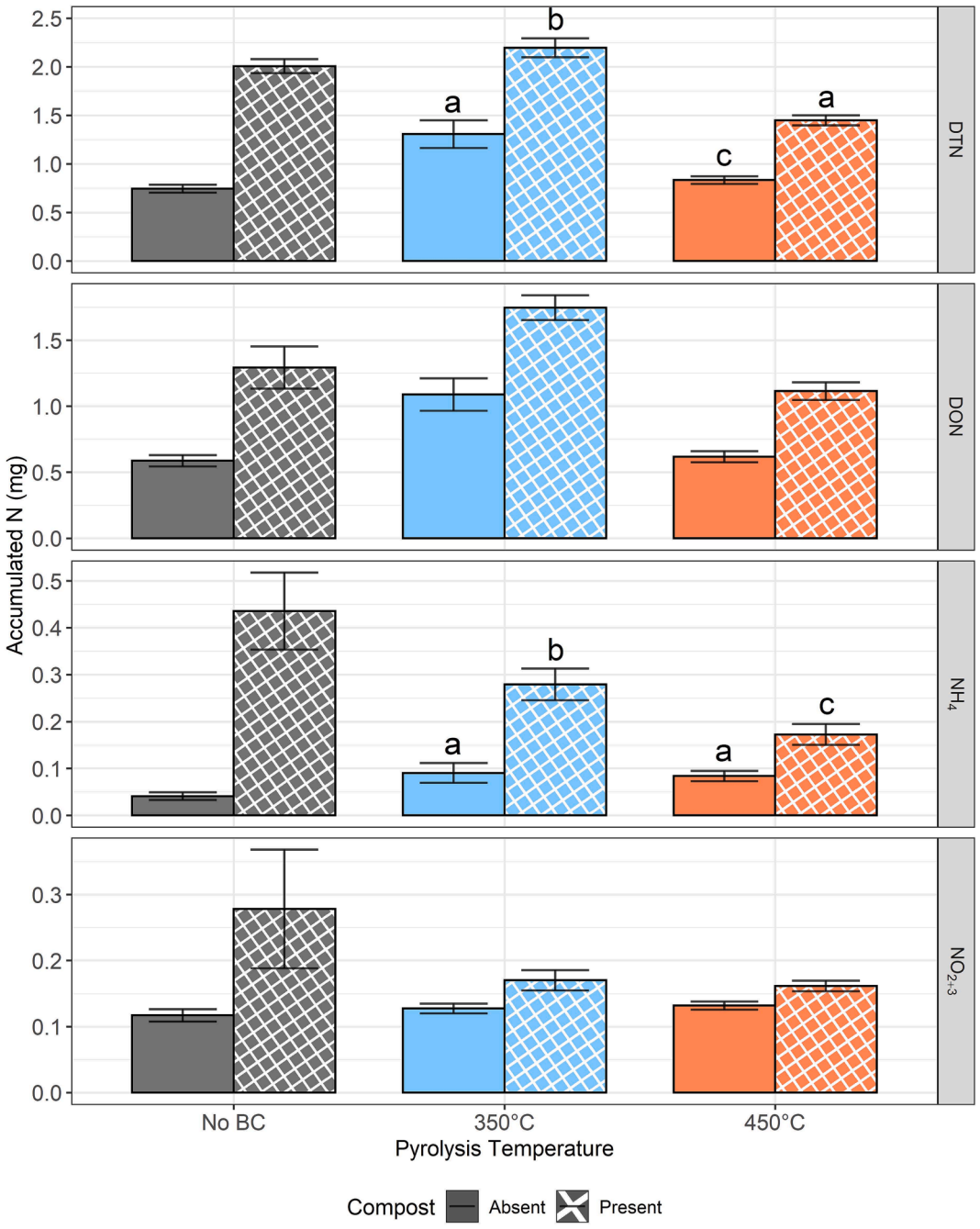

Fig. 4. Dissolved N pyrolysis:compost. Accumulated dissolved N (total mass from each collection extrapolated over volume and summed over 43 days) in the leachate, summarized to show the pyrolysis-compost interaction. Error bars show standard error. Letters indicate significant differences between treatments ($p < 0.05$). Crosshatched and solid bars represent treatments with and without compost respectively.

10% to forest soil. Similarly, Mitchell et al. (2015) measured elevated CO_2 with a 5 t ha^{-1} application rate of sugar maple biochar pyrolyzed at 500 °C. When Sun et al. (2014) applied wheat straw biochar pyrolyzed at 450 °C to a forest soil at a rate of 30 t ha^{-1}, they measured a 30% decrease in CO_2 fluxes, but saw a 7% increase in CO_2 when the same biochar was applied to an agricultural soil. None of these studies used more than one pyrolysis temperature. Other studies suggest that the application of biochar is likely to increase CO_2 emissions in early stages, especially low temperature biochars (250–400 °C), but it may subside with time as abiotic emissions decline (Cross & Sohi, 2011; Zimmerman et al., 2011). Our results indicate that woody biochar pyrolyzed at 350 °C could slightly increase CO_2 efflux from a temperate forest soil, but if the pyrolysis temperature is increased to 450 °C fluxes will either not be affected or may decrease slightly, resulting in an overall larger net increase in soil C. This was especially prevalent for the black ash feedstock which had the greatest CO_2 efflux.

As with the gaseous C fluxes, the DOC response varied with pyrolysis temperature and feedstock. Past studies typically focused on gaseous C fluxes, and only a few have assessed effects on dissolved and particulate leaching (Godlewska et al., 2021), which could potentially be a significant pathway of C loss especially in coarse textured forest soils. In general, the dissolved organic matter associated with biochar reduces in quantity and becomes increasingly protein- and humic-like at higher pyrolysis temperatures (He et al., 2021). Therefore, lower temperature biochar would possess higher quantities of DOM, that potentially would be more easily degraded as was seen in our data. Like CO_2 efflux, the

Table 5
ANOVA results for other leachate metrics. Summary of F-statistic probabilities and degrees of freedom for three-way ANOVA results for leachate models (total accumulated mass) for pH and oxidation reduction potential (ORP) using the 2x2x2 analysis approach (no control) and the two-way ANOVA results for the 5x2 (with control) analysis approach.

		pH	ORP
Model Term	Num DF	p-value	p-value
2x2x2 analysis			
Feedstock	1	0.1240	0.0643
Pyrolysis	1	0.0002	0.7768
Compost	1	0.0077	<0.0001
Feedstock:Pyrolysis	1	0.9186	0.0335
Feedstock:Compost	1	0.3100	0.8068
Pyrolysis:Compost	1	0.4218	0.0055
Feedstock:Pyrolysis:Compost	32	0.0942	0.6141
5x2 analysis			
Biochar	4	0.0003	0.1545
Compost	1	0.0632	<0.0001
Biochar:Compost	4	0.0417	0.1056

Table 6
Mean leachate ORP. Mean oxidation reduction potential values with standard error, summarized to show the feedstock-pyrolysis (top) and pyrolysis-compost (bottom) interactions. Superscript letters indicate treatment differences ($p < 0.05$).

Interactions				
Feedstock	Pyrolysis	n	Mean (mV)	SE
Control	No Biochar	70	246.3	3.4
Balsam Fir	350 °C	70	251.4[a]	2.4
	450 °C	70	247.4[ab]	2.6
Black Ash	350 °C	70	242.7[b]	1.8
	450 °C	70	248.0[ab]	2.7
Pyrolysis	Compost			
Control	Absent	35	257.1	3.4
	Present	35	235.5	5.4
350 °C	Absent	70	252.0[a]	2.2
	Present	70	242.1[b]	1.9
450 °C	Absent	70	258.7[a]	2.3
	Present	70	236.6b[b]	2.3

Table 7
ANOVA results for post-incubation soil chemistry. Summary of F-statistic probabilities and degrees of freedom for three-way ANOVA results for extractable N (TEN) and total N (TN) and C using the 2x2x2 analysis approach (no control) and the two-way ANOVA results for the 5x2 (with control) analysis approach.

		TEN	TN	C
Model Term	Num DF	p-value	p-value	p-value
2x2x2 analysis				
Feedstock	1	0.1972	0.0042	0.0839
Pyrolysis	1	0.0088	<0.0001	0.1441
Compost	1	<0.0001	<0.0001	0.0907
Feedstock:Pyrolysis	1	0.2536	0.0317	0.0442
Feedstock:Compost	1	0.0904	0.2170	0.6534
Pyrolysis:Compost	1	<0.0001	0.5097	0.3085
Feedstock:Pyrolysis:Compost	32	0.1400	0.0019	0.6916
5x2 analysis				
Biochar	4	0.0001	<0.0001	<0.0001
Compost	1	<0.0001	<0.0001	0.0227
Biochar:Compost	4	0.1748	0.0211	0.7429

350 °C biochar had greater DOC leaching compared to 450 °C and the control, but this response was greater in the black ash feedstock than with balsam fir (Fig. 2). For both feedstocks, DOC remained low in the 450 °C biochar treatment relative to the control, indicating that C is relatively stable and not readily dissolved into soil solution. The greater DOC loss from the black ash feedstock at 350 °C is interesting, possibly caused by additional metal–carbon interactions from the higher ash content during pyrolysis [resulting in lower stable polycyclic aromatic carbon (SPAC) formation (e.g. McBeath et al., 2015)] and reduced mineralization due to the lower O:C ratio than the balsam fir biochar at the lower temperature (Table 2). Additional investigations into physical particle strengths were not conducted here but would have been useful in determining the mechanisms for the higher DOC leaching from the lower temperature black ash biochar. These results support our hypothesis that the C response differed by pyrolysis temperature, but some differences were noted between woody feedstocks.

4.2. Nitrogen

We assessed N cycling by measuring N_2O gas fluxes, N species in the leachate, total soil N before and after the incubation, and KCl extractable N at the end of the experiment. The native forest soil used in the experiment had only 0.043% (±0.003%) total N and the compost had 0.48% N. Our finding of no treatment effect on N_2O efflux aligns with other studies that reported no change or slight decreases in N_2O fluxes with biochar application in forest soils (Gogoi et al., 2019; Lin et al., 2017; Malghani et al., 2013; Sackett et al., 2015). One potential mechanism for the reduction in N_2O efflux observed in some studies is the increased soil aeration cause by applying biochar and a decrease in the number of micro-sites suitable for denitrification (Gogoi et al., 2019). Thangarajan et al. (2018) suggests biochar can reduce N_2O emissions via NH_3^- absorption, reducing the pool of NH_4^+ available for nitrification, and direct nitrification inhibition by biochar. In this laboratory incubation, we measured positive, but low N_2O fluxes regardless of treatment. Total cumulative N ranged from about 1 g m^{-2} to <4 g m^{-2} over 42 days in the incubation. These low rates are likely a result of low soil N coupled with well-aerated conditions expected of a coarse-textured soil and are likely the primary reason why no effect of the treatments was observed.

Dissolved N showed a similar response to the treatments as DOC, where more N was leached from the 350 °C pyrolysis treatment than the 450 °C pyrolysis treatment. The 450 °C biochar seemingly prevented N loss following N fertilization by compost as shown by the reduction in DTN in the leachate relative to the control (Figures 4, S3). This response was largely driven by differences in DON with some contribution from NH_4 for the black ash feedstock treatment and none from nitrate (Figs. 3, 4). Given that N content in the 350 °C biochar was similar or lower than the 450 °C biochar, these differences between treatments can largely be attributed to changes in microbial N processing rather than additional N inputs associated with the biochar. Indeed, for balsam fir, N content in the 450 °C biochar was almost double that of the 350 °C biochar, highlighting the ability of high temperature biochars to retain added N.

In the post-incubation soil extraction, NH_4 and NO_{2+3} contributed equally to total extractable N. Unsurprisingly, the addition of compost increased the amount of TEN, however, the amount by which it increased differs by pyrolysis temperature. The addition of biochar to compost amended soil decreases TEN, especially at 450 °C where TEN is similar to the control without compost. Thus, the 450 °C biochar is adsorbing and potentially holding onto more N, in the form of NH_4 and NO_{2+3}, than the lower temperature biochar. Biochar alone (no compost) seemed to slightly decrease TEN compared to the control, also indicating that biochar is adsorbing both NH_4 and NO_{2+3} or mobilizing it and increasing N loss through N_2O and leachate fluxes. In this experiment, given the N response in the leachate, biochar is slightly increasing N fluxes, but is primarily adsorbing mineral forms of N. Going further, the 350 °C biochar is mobilizing N and the 450 °C biochar is adsorbing it, supporting our hypothesis that the response would differ with pyrolysis temperature. We did see an increase in N leaching rates over the course of the incubation, so given more time the balance between adsorbing and mobilizing may change as the biochar becomes saturated and microbial communities grow and change, as biochar is altered by aging (Kalu et al., 2021; Kim et al., 2021). Since biochar has the potential to absorb N from the soil, co-composting biochar with nutrient rich

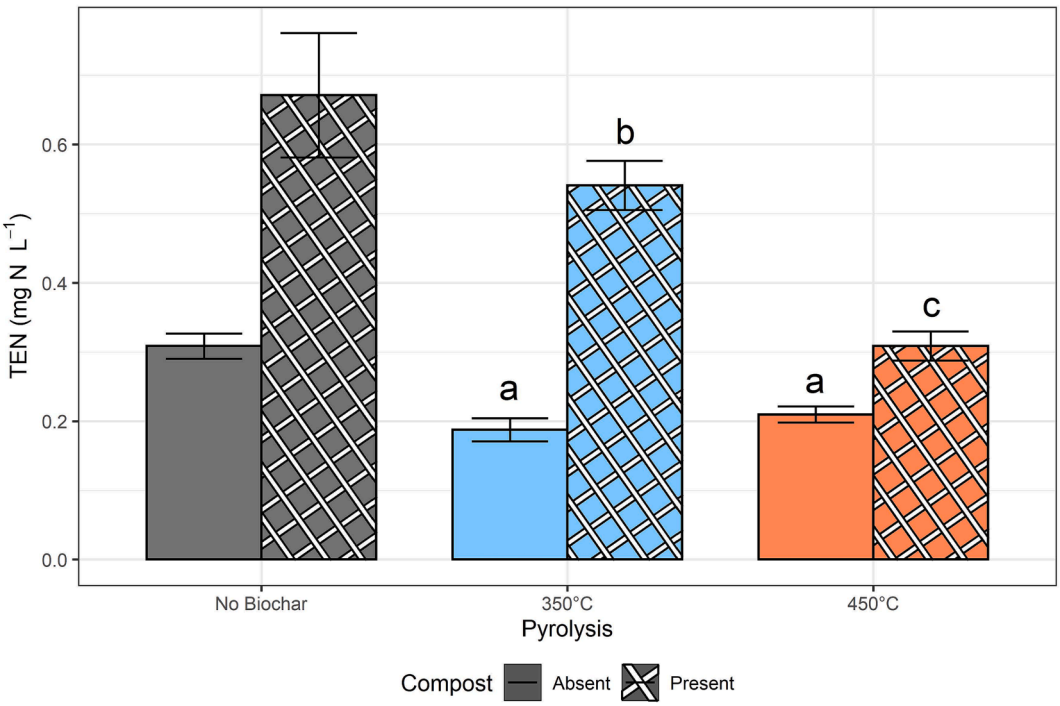

Fig. 5. Post-incubation N. Total extractable N from a post-incubation soil extraction summarized to show the pyrolysis-compost interaction. Error bars show standard error. Letters indicate significant differences between treatments ($p < 0.05$). Crosshatched and solid bars represent treatments with and without compost respectively.

biowaste prior to application may ensure the soil amendment is a nutrient source, if that effect is desired (Khan et al., 2016).

4.3. Biochar properties

The pH of the biochar itself was higher for the 450 °C biochar than the 350 °C biochar in both feedstocks, which is consistent with other studies (Ahmad et al., 2012; Al-Wabel et al., 2013; Hagner et al., 2016). When measuring the pH of the leachate, the 450 °C biochar was also slightly, but significantly higher than the 350 °C biochar in the absence of compost, but neither biochar groups were different from the control. The pH of the leachate generally increased over the incubation and was typically higher than the pH of the biochar alone. The slight increase in pH indicates the potential to use biochar as a liming agent in a soil amendment (Bolan et al., 2022).

The oxidation reduction potential of the leachate decreased over the incubation and was higher than expected. The mean ORP (+247 mV) suggests a well aerated and highly oxic conditions within the soil columns. This could have significantly reduced the amount of N_2O being produced through microbial denitrification (ORP between −50 to 50 mV). The redox capacity of biochar depends on the feedstock, pyrolysis conditions, and any subsequent post-production activation of the biochar, with biochar typically being more reduced than the original feedstock materials (Joseph et al., 2015; Yuan et al., 2017). In this study, biochar had no effect on ORP, but the addition of compost decreased ORP in most cases. This indicates that these biochars did not drastically alter the microbial communities directly or indirectly by changing the redox conditions within the soil columns.

4.4. Conclusion

As with other studies, we found mixed effects of applying biochar to a forest soil (Gogoi et al., 2019), but generally clear effects of pyrolysis temperature, supporting our hypothesis. The lower pyrolysis temperature, 350 °C biochar generally increased the mobilization of C and N with increased CO_2 fluxes and greater N and DOC leaching. In contrast, the higher temperature, 450 °C biochar seemed to adsorb and hold onto nutrients while having little impact on CO_2 fluxes, indicating it may be a suitable amendment to cause a net increase in soil C. The black ash feedstock had greater C leaching than balsam fir, which is intriguing and may indicate that woody feedstocks differ in some stability processes, counter to our hypothesis. But overall, responses were similar across all other metrics suggesting that woody feedstocks produce biochar with similar properties. This experiment builds on the consensus that biochars are a product of their feedstocks, are shaped by the pyrolysis process, and need to be carefully matched with the soil and environment to provide the most benefit while limiting negative impacts.

While the laboratory incubation has limited inference to field conditions, the controlled experiment allowed us to examine the mechanisms and interactions between a nutrient-poor, temperate forest soil and different types of biochar. After assessing the influence of biochar on a native forest soil, it is possible and important to take the next step and apply biochar in a field study including trees. In particular, it would be useful to determine if a higher pyrolysis biochar (e.g., produced > 450 °C) could have neutral or positive effects on tree growth, resulting in a net increase in soil C storage. Continued research of biochar application in practical settings will help us to understand if there is a place for biochar in forestry to combat climate change, enhance tree survival and growth, and remove forestry byproducts from the waste stream.

CRediT authorship contribution statement

Alan J.Z. Toczydlowski: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Robert A. Slesak:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Rodney T. Venterea:** Conceptualization, Methodology, Resources, Writing – review & editing. **Kurt A. Spokas:** Conceptualization, Investigation, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

USDA is an equal opportunity lender, provider, and employer. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR). We gratefully acknowledge Gwendolen Keller for assistance with data collection, Douglas Brinkman for growth chamber management, and Scott Mitchell and Martin DuSaire for sample analysis.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2023.120881.

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