



Towards predicting biochar impacts on plant-available soil nitrogen content

Claire L. Phillips¹ · Kylie M. Meyer² · Manuel Garcia-Jaramillo³ · Clara S. Weidman⁴ · Catherine E. Stewart⁵ · Thomas Wanzek⁴ · Michael A. Grusak⁶ · Donald W. Watts⁷ · Jeff Novak⁷ · Kristin M. Trippe^{2,4}

Received: 14 September 2021 / Accepted: 16 December 2021
© The Author(s) 2022

Abstract

Biochars can improve soil health but have been widely shown to reduce plant-available nitrogen (N) owing to their high carbon (C) content, which stimulates microbial N-immobilization. However, because biochars contain large amounts of C that are not microbially available, their total elemental C:N ratio does not correspond well with impacts on soil N. We hypothesized that impacts on soil plant-available N would relate to biochar mineralizable-C (C_{\min}) content, and that C:N ratios of the mineralizable biochar component could provide a means for predicting conditions of net soil N-mineralization or -immobilization. We conducted two laboratory experiments, the first measuring biochar C_{\min} from respiration of isotopically labeled barley biochars manufactured at 300, 500, and 750 °C, and the second characterizing C_{\min} by proxy measurements for ten biochars from six feedstocks at several temperatures. For both experiments, soils were incubated with 2% biochar by mass to determine impacts to soil N-mineralization. Contrary to expectation, all the biochars increased soil N-mineralization relative to unamended soils. Also unexpected, higher temperature (500 and 700 °C) barley biochars with less C_{\min} stimulated more soil decomposition and more soil N-mineralization than a 350 °C barley biochar. However, across diverse biochar feedstocks and production methods, none of the biochar characteristics correlated with soil N-mineralization. The finding of improved soil N-mineralization adds complexity to the range of soil N responses that can be expected in response to biochar amendment. Because of the limited ability to predict soil N responses from biochar properties, users should monitor soil N to manage soil fertility.

Highlights

- A diverse set of biochar types all increased net soil N-mineralization in a 4-week aerobic incubation.
- Biochar mineralizable-C and -N did not predict soil N-mineralization across feedstocks.
- For individual feedstocks, soil N-mineralization increased with pyrolysis temperature.

Keywords C:N ratio · Plant-available nitrogen · Priming · Respiration · ¹³C

✉ Claire L. Phillips
claire.phillips@usda.gov

¹ USDA-ARS National Laboratory for Agriculture and Environment, Ames, IA, USA

² USDA-ARS Forage Seed and Cereal Research Unit, Corvallis, OR, USA

³ Department of Environmental Toxicology, Oregon State University, Corvallis, OR, USA

⁴ Department of Crop and Soil Science, Oregon State University, Corvallis, OR, USA

⁵ USDA-ARS Soil Management and Sugarbeet Research Unit, Ft. Collins, CO, USA

⁶ USDA-ARS Edward T. Schafer Agricultural Research Center, Fargo, ND, USA

⁷ USDA-ARS Coastal Plain Soil, Water, and Plant Research Center, Florence, SC, USA

1 Introduction

Biochar incorporation into agronomic systems has become an increasingly popular management practice in the past decade. The addition of biochar has been shown to boost crop yields (Spokas et al. 2012) by altering nutrient cycling (Mia et al. 2017), increasing soil pH (Clough and Condron 2010), and in some cases altering soil water properties (Liu et al. 2017; Phillips et al. 2020a), while additionally sequestering biomass carbon in long-lived forms (Baldock and Smernik 2002; Kuzyakov et al. 2009; Woolf et al. 2010). However, increasing the carbon (C) content of the soil system through biochar application typically increases the C to nitrogen (N) ratio, which in turn impacts soil microbial function and reduces plant-available N (Liu et al. 2018; Gao et al. 2019).

Biochars are highly aromatic, high C materials produced by pyrolysis, gasification, and other low-oxygen heating processes. Many biochars are made from low-value biomass such as wood or straw feedstocks with wide C:N ratios, which confers wide C:N ratios to the resulting biochar. Exceptions are biochars produced from food waste and manures that have narrow C:N ratios. However, N retention during biochar production can also be highly variable, which further contributes to diverse C:N ratios in resulting biochars (Enders et al. 2012). Depending on the feedstock and pyrolysis conditions, biochar can have a total C:N ratio ranging from 6.5 to 640 (Bonanomi et al. 2017).

Several recent meta-analyses (Nguyen et al. 2017; Liu et al. 2018; Gao et al. 2019) showed that reductions in soil mineral N are a widespread response to biochar application. Across approximately 900 studies, Nguyen et al. (2017) found biochar application decreased $\text{NH}_4\text{-N}$ by an average of 11% and $\text{NO}_3\text{-N}$ by 10%. Evidence that microbial immobilization caused this decline in $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ (collectively dissolved inorganic nitrogen, DIN) was provided by reports of increased CO_2 emissions and increases in microbial abundances associated with N cycling, indicating increased microbial N demand following biochar application (Deenik et al. 2010; Zhang et al. 2012; Zheng et al. 2012; Ducey et al. 2013).

Initial decomposition of residues with wide C:N ratios usually results in N-immobilization in soil (Vigil and Kissel 1991; Aulakh et al. 1991). In some cases a critical C:N threshold can be used to predict conditions of N-mineralization or N-immobilization in soil (Trinsoutrot et al. 2000; Andrews and Foster 2007), although such thresholds are also dependent on residue quality (Ågren et al. 2013; Manzoni 2017). In the case of biochar, a critical C:N ratio ranging between 20 and 32 for biochar and over 32 for the soil as a whole has been shown to support microbial immobilization of N (Nguyen et al. 2017). However, we expected that the C and N content of the easily mineralizable fraction of biochar

would have a better ability than total biochar C:N to predict N immobilization, because a large portion of biochar C is highly resistant to microbial decay. As pyrolysis temperature is increased much of the carbon becomes heterocyclic and largely inaccessible for microbial decomposition (Budai et al. 2016; Leng et al. 2019). Although the quantity of total C in biochar generally increases with pyrolysis temperature, the quantity of mineralizable-C (C_{min}) decreases, due to reduction of organic molecules into aromatic rings and loss of COOH, ROH, and C=N functional groups (Keilueit et al. 2010; Torres-Rojas et al. 2020). The resistance of biochars to microbial decomposition has been demonstrated by numerous incubation studies. A meta-analysis of incubation studies showed that as much as 15% of biochar carbon decomposed within two years, but that on average the labile pool accounted for just 3% of total C (Wang et al. 2016). Wang et al. (2016) furthermore estimated the inert biochar C pool to have a residence time of over 500 years. Biochar effects on N availability are therefore likely to be associated with only the portion of biochar C that is easily mineralizable (Jeffery et al. 2015; Gao et al. 2019).

In this study we employed biochars from multiple feedstocks, pyrolysis temperatures, and production technologies to examine links between the mineralizable-C (C_{min}) content of biochars, the C:N ratio of mineralizable component, and resulting impacts on net soil N-mineralization. We hypothesized that: (1) biochar C_{min} would correlate negatively with soil N-mineralization, because most biochars would be poor sources of N, and higher quantities of C_{min} would therefore add more low-N substrate to soil. We further hypothesized that (2) the C:N ratio of the mineralizable component of biochar, estimated from water-extractable organic C and N concentrations, would also be negatively correlated with soil N-mineralization.

2 Materials and methods

Two experiments were conducted to evaluate the hypotheses. The first experiment used a temperature series of ^{13}C -labeled barley biochars produced at 350, 500, and 700 °C. C_{min} was quantified by isotopic analysis of respired CO_2 in a 101-day soil incubation. Soil DIN concentrations were simultaneously measured to determine net N-mineralization. To expand to more biochar types, the second experiment examined non-isotopic measures of C_{min} for ten biochars produced from several feedstocks under a range of temperatures and several production technologies. C_{min} was estimated by several approaches, including H:C elemental ratio which provides a measure of biochar aromaticity (Budai et al. 2013), water extractable organic C (WEOC) which estimates one of the labile pools (Whitman et al. 2014), and

permanganate-oxidizable (KMnO_4 -oxidizable) C which estimates oxidation resistance (Calvelo Pereira et al. 2011). Net N-mineralization in biochar-amended soils was determined in a 28-day aerobic soil incubation.

2.1 Experiment 1

2.1.1 Biochar and soils

A set of barley (*Hordeum vulgare* L.) whole-plant biochars was produced by pyrolysis at 350, 500, and 700 °C from a mixture of ^{13}C -labeled panicles and leaves of barley and non-labeled barley straw. The ^{13}C -labeled barley was grown in a controlled environmental chamber and pulse-labeled 3 times with $^{13}\text{CO}_2$ to achieve ^{13}C enrichment of 0.38 atom percent excess, as described by Lifschitz et al. (2002). Due to limited quantities of this material, it was diluted with biochar made from non-labeled barley straw grown near Corvallis, Oregon. The labeled and non-labeled plant materials were ground to pass through a 6 mm sieve, pyrolyzed separately, and then mixed in a ratio of 20% labeled to 80% non-labeled material by mass, providing $\delta^{13}\text{C}$ values for the mixtures of 32–40‰ PDB (Table 1). The biochars were pyrolyzed using a Lindburg oven with a retort at the USDA-ARS in Florence, South Carolina as outlined by Cantrell and Martin (2012). Chemical characteristics of these biochars, as well as the biochars used in Experiment 2, are shown in Table 1 and testing methods are detailed below.

The barley biochars were added at a rate of 2% by mass to two silt loam soils with contrasting organic carbon contents. A total of 8 treatments were examined: each of the three biochars was added to a low-carbon and a high-carbon soil and compared to unamended soils as controls. The higher carbon content soil was classified as fine-silty, mixed, superactive, mesic Cumulic Ultic Haploxeroll in the ‘Chehalis’ series, and the lower carbon content soil was classified as a fine-silty, mixed, superactive, mesic Pachic Ultic Argixeroll in the ‘Willamette’ series (Soil Survey Staff 2006). Additional soil characteristics are shown in Table 2. Both soils were collected from 0–15 cm depth from research farms near Corvallis, OR. Soils were sieved through a 4 mm mesh to homogenize and stored at 4 °C for 5 days prior to incubations.

2.1.2 Incubations and isotopic measures

Soil-biochar mixtures were incubated for 101 days and sampled for respired CO_2 and $\delta^{13}\text{CO}_2$ on days 1, 2, 3, 4, 7, 14, 21, 32, 42, 57, 73, and 101. Seventy-five g samples (dry-mass equivalent) of soil or soil-biochar mixture were measured into graduated polyethylene specimen cups and wetted with 0.01 M CaCl_2 to bring to 60% water-filled pore space. Three replicates of the eight treatments were prepared, for

a total of 24 samples. The polyethylene cups were placed inside 1.8 L glass canning jars; 10 mL of water was added to the jars outside the cups to maintain humidity; and the jars were sealed. The headspace was flushed by piercing rubber septa installed in the lids and flowing CO_2 -free air through at a rate of 500 mL min^{-1} for 15 min. Process standards for CO_2 measurements were prepared by flushing empty jars with CO_2 -free air or a 4000 ppm CO_2 standard. Standard jars were incubated alongside soils to detect and quantify air leaks. Jars were incubated at 23 °C in a darkened environmental chamber between sampling events.

At each sampling event, a subsample of headspace air was collected for $\delta^{13}\text{CO}_2$ by removing 12 mL of air with a gastight syringe and injecting it into a borosilicate vial (Extetainer®, Labco Ltd.) that had been purged with CO_2 -free air and pre-evacuated. Headspace CO_2 concentration was then measured by connecting one of the jar’s septa to a CO_2 analyzer (Ultraportable Greenhouse Gas Analyzer, Los Gatos Research) and drawing headspace air for 2 min. Data measured between 30 and 100 s were averaged. The jar headspace was then flushed with CO_2 -free air to prepare for the next phase of CO_2 accumulation. Respiration rate was computed as the g C- CO_2 accumulated, divided by the time between each sampling event. Exetainer vials were accumulated over the study and shipped in batches after days 42 and 101 to Ft. Collins, Colorado, where $\delta^{13}\text{CO}_2$ was determined on a gas chromatograph-isotope ratio mass spectrometer (Isoprime Inc., Manchester, UK).

Quantities of CO_2 derived from soil and biochar were calculated using a 2-member mixing model, as follows:

$$f_{\text{biochar}} = \frac{\delta_{\text{mixture}} - \delta_{\text{soil}}}{\delta_{\text{biochar}} - \delta_{\text{soil}}} \quad (1)$$

where f_{biochar} is the fraction of respiration from biochar, δ_{mixture} is the $\delta^{13}\text{CO}_2$ respired from the biochar-soil mixture, and δ_{soil} and δ_{biochar} are the $\delta^{13}\text{CO}_2$ respired from soil and biochar end-members, respectively. The end-members were estimated as the $\delta^{13}\text{CO}_2$ respired from unamended soil, and the $\delta^{13}\text{C}$ of the solid biochars.

2.1.3 Soil N-mineralization

Soil net N-mineralization was quantified in accompanying soil incubations that were destructively extracted for N-NO_3^- and N-NH_4^+ (collectively DIN) on days 0, 1, 2, 3, 4, 7, 14, 21, 42, and 101. The incubations for N-mineralization were prepared in the same way as for C_{min} measurements. To ensure the samples remained aerated during incubation, each jar was manually vented twice weekly by briefly removing the lid for a few seconds. Three replicates of the 8 treatments were prepared for each of the 9 sampling events through day 42. The jars used for respiration measurements

Table 1 Production conditions and chemical characteristics of biochars

Variable	Production Method												
	Pyrolysis												
	Barley plant			Grape wood			Poultry litter			Flame-cap pyrolysis			
Temperature (°C)	350	500	700	350	700	700	350	700	350	700	Juniper wood	Douglas fir wood	Wheat straw
$\delta^{13}\text{C}$ (‰ PDB)	39.7	37.1	32.0										
Total Carbon (%)	65.5	67.7	69.7	71.3	83.5	42.3	47.2	83.9	83.9	79.7	79.7	56.0	56.0
Total H (%)	4.77	3.43	1.89	4.65	1.90	0.84	3.83	1.41	1.41	0.66	0.66	1.13	1.13
Total N (%)	1.18	1.19	1.19	0.97	1.01	2.02	5.22	0.57	0.57	0.38	0.38	0.66	0.66
C:N (atomic)	64.6	66.4	68.6	85.7	96.5	24.5	10.5	172.7	172.7	246.8	246.8	98.9	98.9
H:C (atomic)	0.88	0.61	0.33	0.78	0.27	0.24	0.97	0.20	0.20	0.10	0.10	0.24	0.24
Ash (%)	13.9	17.7	22.1	7.1	10.5	53.2	33.6	18.1	18.1	5.4	5.4	51.3	51.3
pH	10.2	10.0	11.2	8.0	10.7	11.1	8.3	8.1	8.1	9.7	9.7	10.8	10.8
EC (mS cm ⁻¹)	3.63	3.92	6.95	1.34	1.96	12.81	9.35	0.22	0.22	0.43	0.43	6.33	6.33
CaCO ₃ equiv. (%)	11.7	13.8	15.9	9.0	22.1	62.2	30.5	6.4	6.4	8.8	8.8	26.3	26.3
Inorganic C (mg g ⁻¹)	14.7	100.3	131.4	8.7	107.0	94.1	17.9	16.5	16.5	23.1	23.1	90.1	90.1
Extract. Ca ^a (mg kg ⁻¹)	215	226	227	12,903	17,460	51,313	34,557	7165	7165	6889	6889	3157	3157
Extract. K ^a (mg kg ⁻¹)	1215	1358	13,720	11,927	17,733	97,260	58,990	2562	2562	8991	8991	30,935	30,935
Extract. Mg ^a (mg kg ⁻¹)	37	34	31	2304	2050	16,693	11,833	699	699	1123	1123	965	965
Extract. Na ^a (mg kg ⁻¹)	14	16	22	13	233	29,823	19,167	562	562	324	324	3753	3753
Extract. S ^a (mg kg ⁻¹)	81	89	58	0	0	2228	8997	185	185	4	4	521	521
Extract. P ^b (mg kg ⁻¹)	1302	1586	1548	745	1000	36,130	25,437	136	136	2239	2239	2262	2262
NO ₃ -N ^c (mg kg ⁻¹)	0	0	0	0	0	0	0.0	78.9	78.9	0	0	0	0
NH ₄ -N ^c (mg kg ⁻¹)	0.5	0.8	0.4	2.5	0.0	3.2	20.7	0.0	0.0	0.6	0.6	0.4	0.4
TDN (mg kg ⁻¹)	70	17	10	74	6	98	4665	224	224	2	2	19	19
WEOC (mg kg ⁻¹)	833	247	503	12,455	591	510	16,414	322	322	293	293	846	846
WEOC:TDN	12.0	14.6	52.0	168.2	96.8	5.2	3.5	1.4	1.4	156.8	156.8	44.4	44.4
KMnO ₄ -oxidizable C (mg kg ⁻¹)	9.11	2.61	1.59	0.61	0.59	2.37	1.45	0.97	0.97	2.12	2.12	0.79	0.79

Experiment 1 used barley plant biochars only, and experiment 2 used all the biochars. Values are averages, $N=2$ for $\delta^{13}\text{C}$ and $N=3$ for all other measures

EC electrical conductivity, TDN total dissolved nitrogen, WEOC water-extractable organic carbon

^a1 M HCl extract

^b2% formic acid extract

^c2 M KCl extract

Table 2 Cropping history and select physiochemical characteristics of two soils collected near Corvallis, Oregon ($N=3$)

Variable	Chehalis	Willamette
Crop history	Organic vegetables	Grass seed, small grains
Sand-silt-clay ^a (%)	7-58-35	7-72-21
$\delta^{13}\text{C}$ (‰ PDB)	- 27.1	- 27.8
Total C (%)	3.7	1.7
Total N (%)	0.25	0.13
C:N	14.72	13.31
$\text{NO}_3\text{-N}^{\text{b}}$ (mg kg^{-1})	2.91	0.78
$\text{NH}_4\text{-N}^{\text{b}}$ (mg kg^{-1})	0.88	0.53
$\text{pH}_{2:1}$	6.82	6.48

Organic carbon was assumed to equal total carbon, owing to low soil pH

^aTypical values for A-horizon from soil survey

^b2 M KCl extraction

were destructively sampled on day 101, providing a total of 240 DIN samples.

Soil DIN was measured on a moist subsample (approx. 7.5 g air-dried equivalent) extracted in a 1:4 w/v ratio with 2 M KCl. Soils were shaken for 1 h with extraction solution, and then filtered through Whatman #1 filters. The filtrate was analyzed colorimetrically using the vanadium-chloride method for NO_3 (Doane and Horwath 2003) and the salicylate-nitroprusside method for NH_4 (Rhine et al. 1998) with a 96-well plate spectrophotometer (BioTek Instruments). Soil DIN concentrations were corrected for moisture content, determined from oven-dry (105 °C, 24 h) sub-samples of the incubated soils. Net N-mineralization on each sampling day was calculated as the difference between average DIN for that day and for day 0 baseline samples.

2.1.4 Soil and biochar fertility

The two soils were characterized for pH (2:1 water to soil) on oven-dried (105 °C), sieved samples. Ground samples were also analyzed for total C and N by combustion-IR (CN Analyzer, LECO Corporation). Biochars were characterized for extractable nutrients, EC, pH, and liming ability as potential co-variates of N-mineralization. Extractable nutrients were determined on oven-dried (105 °C) and ground biochar following the method recommendations of Camps-Arbestain et al. (2015). Available-P was determined from a 2% formic acid extraction (Wang et al. 2012; after Rajan et al. 1992) with concentrations determined using inductively coupled plasma optical emissions spectrometry (ICP-OES, Perkin-Elmer Optima 4300 DV). Available-K, Ca, Mg, Na, and S were determined from a 1 M HCl extraction (Camps-Arbestain et al. 2015 from Rayment and Lyons

2011), followed by ICP-OES analysis. Liming equivalence ($\text{CaCO}_3\text{-eq}$) was determined by reacting the biochar with 1 M HCl followed by titration with 0.5 M NaOH (Rayment and Lyons 2011). Biochar N-NO_3^- and N-NH_4^+ were measured by extracting 1 g biochar with 2 M KCl in a 1:10 w/v ratio and analyzing the extract colorimetrically as for soil. Biochar pH and EC were determined from a 1:20 biochar-water solution.

Inorganic carbon content was determined by measuring CO_2 evolved from an acidified sample of dry (non-incubated) biochar, similar to the method of Calvelo Pereira et al. (2017). A 1 g oven-dry (105 °C) biochar sample was placed in a glass beaker, which was sealed in a 1.8 L canning jar, and the headspace was flushed with CO_2 -free air. Using a syringe, 5 mL water and 10 mL 2 M HCl were added to the biochar via a septum in the jar lid attached to a drainage tube. After waiting 24 h for the sample to completely react, CO_2 production was measured by connecting the jar to a CO_2 analyzer, as described previously for soil incubations.

2.2 Experiment 2

Seven non-isotopically labeled biochars were studied in addition to the three barley plant biochars to evaluate C_{min} and soil N relationships for a greater range of biochar types (Table 1). The non-labeled biochars included: grape wood (*Vitis vinifera* L.) prunings pyrolyzed at 350 and 700 °C at the USDA-ARS in Florence, South Carolina (Cantrell and Martin 2012); poultry litter obtained from a commercial source (Stutzman Environmental Products, Inc.), also pyrolyzed at 350 and 700 °C in Florence; a juniper wood (*Juniperus occidentalis* Hook.) biochar produced by flame-cap pyrolysis at > 1250 °C (Phillips et al. 2020b), and mixed-conifer wood and wheat straw (*Triticum aestivum* L.) biochars, both produced by gasification at > 1250 °C as described by Phillips et al. (2020a).

Three non-isotopic assessments of biochar C_{min} were performed on these biochars, including (1) H/C atomic ratio, (2) water-extractable organic carbon (WEOC) content, and (3) permanganate-oxidizable (KMnO_4 -oxidizable) carbon content. Biochar total C, H, N, and ash content were measured by an external laboratory (Mineral Labs Inc., Salyersville, Kentucky). WEOC and total dissolved nitrogen (TDN) were determined from a 5.0 g sample extracted with 50 mL deionized water, as suggested by Chinu et al. (2017), followed by analysis using a TOC analyzer with automated acidification to remove inorganic C (TOC-VCPN, Shimadzu Scientific Instruments). KMnO_4 -oxidizable C was determined in the same way as the soil method described by Weil et al. (2003), but using only 0.15–1.0 g of biochar owing to its high carbon content.

Biochar impacts on soil N-mineralization were determined by incubating each of the ten biochars with the Willamette soil, using a fresh sample of the Willamette soil collected in spring 2019. 75 gram samples of soil, either unamended or amended with 2% biochar by mass, were prepared for aerobic incubations in the same way as experiment 1. Samples were incubated at 23 °C in a darkened environmental chamber, and three replicates of each treatment were destructively sampled on days 14 and 28 following Drinkwater et al. (1996). In addition, three replicate 10 g samples were prepared for each treatment as a day 0 baseline sample. Soils were analyzed for N-NO_3^- and N-NH_4^+ as described for experiment 1. Mineralized-N on days 14 and 28 were assessed as the difference between DIN on those days and day 0 baseline samples.

2.3 Statistical analysis

For experiment 1, cumulative soil respiration, biochar-C respiration, and soil N-mineralization were analyzed by two-way analysis of variance (ANOVA) to test whether responses differed by soil type or biochar pyrolysis temperature, using the base package of R (R Core Team 2020). Tukey's honest significant difference test was used for post-hoc tests of pairwise treatment differences. Linear regression was also used to test whether soil N-mineralization correlated with biochar respired-C, alone or normalized by biochar total-N content. Biochar respired-C, soil type, and their interaction were initially evaluated as explanatory variables, and the non-significant interaction term was subsequently excluded.

For the larger set of biochars in experiment 2, regression analysis was again used to test whether soil N-mineralization correlated with KMnO_4 -oxidizable C, H:C ratio, WEOC, and WEOC:TDN. Averages of three analytical replicates for each biochar type were used for regressions. All of the measures of C_{\min} with the exception of H:C were visually determined to have long-tailed distributions (i.e., a few high values) and were log-transformed.

A principal component analysis (PCA) was performed on the dataset from experiment 2 to evaluate what qualities distinguished the biochars from one another, and to evaluate what characteristics corresponded with stimulation of soil N-mineralization. The initial dataset included all variables shown in Table 1. To stabilize the results and reduce ordination complexity, the large number of explanatory variables were reduced to nine, one fewer than the number of biochars analyzed. Some groups of variables that represented similar functions were reduced (e.g., inorganic-C, pH, and ash), and variables that made small contributions to the first three principal components were also omitted. Long-tailed data were log-transformed, and data were centered and scaled to normalize variances. PCA was performed

with the 'FactoMineR' (Husson et al. 2020) and 'factoextra' (Kassambara and Mundt 2020) packages in R.

3 Results

3.1 Experiment 1: Barley biochar temperature series impacts on soil C- and N-mineralization

The respired-C from barley plant biochars decreased as pyrolysis temperature increased, thus demonstrating lower quantities of C_{\min} at higher pyrolysis temperatures (Fig. 1a). The 350, 500, and 700 °C biochars respired approximately 18, 6, and 4% of their original C contents over the 101-day incubation, respectively, with similar amounts respired in both soils.

Biochar amendments also had positive priming effects, increasing soil C respiration relative to unamended soil (Fig. 1b). In the Willamette soil, all the biochars caused significantly more soil respiration than unamended soil ($p \leq 0.02$ for each pairwise comparison), and the strength of the priming effect increased with biochar pyrolysis temperature. Cumulative 101-day soil respiration increased by 20, 35, and 54% for the 350, 500, and 700 °C biochars, respectively, compared to unamended soil. In the high-C Chehalis soil, the priming effect was only significant for the 700 °C biochar, amounting to a 34% increase in cumulative respiration relative to the control ($p = 0.002$).

Rates of N-mineralization also increased with pyrolysis temperature (Fig. 2). Soils with no biochar or the 350 °C biochar initially immobilized N in the first days to weeks of incubation, while soils with 500 or 700 °C had net mineralization throughout the incubation (Fig. 2). Treatment differences were greatest on measurement day 42 and later converged. Focusing on day 42 to characterize treatment effects (Fig. 2 inset plots, and Fig. 3), the 350 °C biochar decreased net N-mineralization compared to unamended soil, although the effect was only significant in the Chehalis soil. The 500 and 700 °C biochars increased net N-mineralization in the Willamette soil, and were similar to the unamended control in the Chehalis soil.

Figure 3 shows the relationship between cumulative soil N-mineralization and cumulative soil-C respired by day 42, relative to unamended soils. Overall, the 500 and 700 °C biochars tended to cause more positive priming of soil respiration and to stimulate more soil N-mineralization, while the 350 °C biochar had a smaller positive priming effect and immobilized N. While there was not a linear trend of more soil N-mineralized as more soil-C was respired, the biochars segregated into quadrats. The 350 °C biochar fell in the lower right quadrat—priming soil C and immobilizing soil N—while the 500 and 700 °C biochars generally fell in

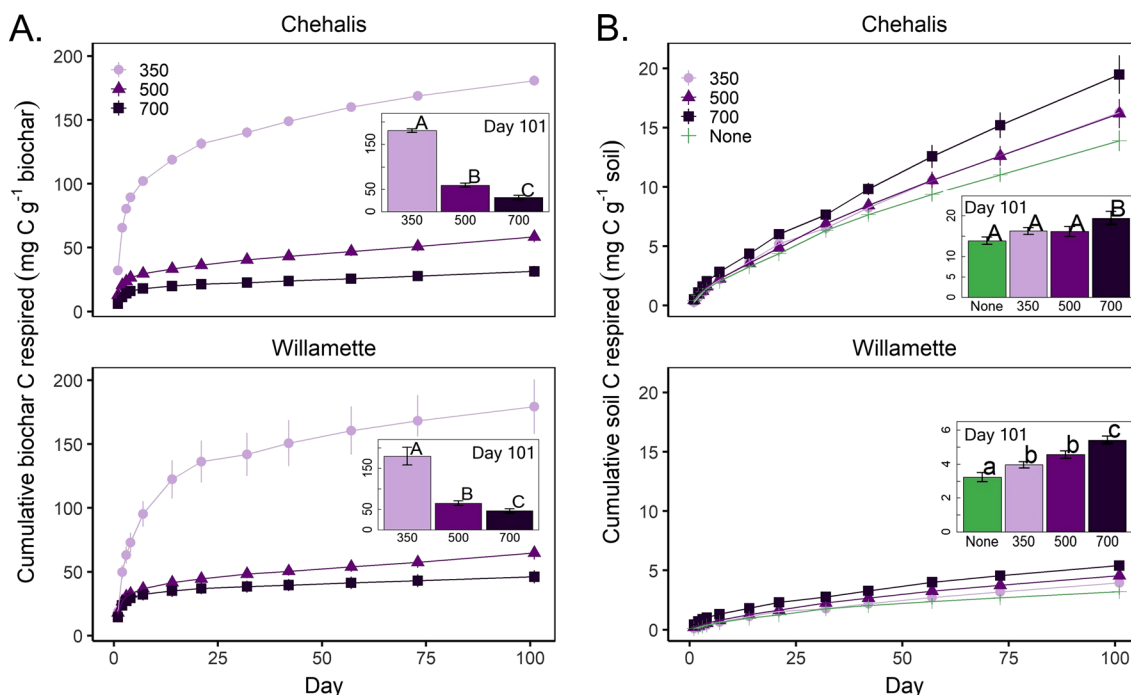


Fig. 1 A Timeseries of respired C from barley biochar relative to total biochar C and B respired soil C relative to total soil organic C during a 101-day incubation. Inset bar charts show cumulative respiration on day 101, where treatments sharing letters of the same case had non-

significant differences ($p > 0.05$). The Chehalis (3.7% C) and Willamette (1.7% C) soils were collected near Corvallis, Oregon, USA. Biochars were pyrolyzed at 350, 500, or 700 °C. Error bars show standard deviation ($N = 3$)

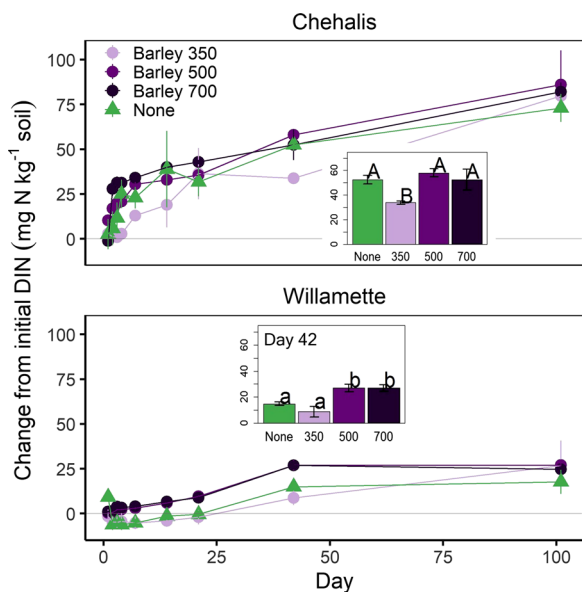


Fig. 2 Timeseries of dissolved inorganic nitrogen (DIN) concentrations over a 101-day incubation, with day 0 baseline concentrations subtracted. Inset bar charts show net N mineralized in 42 days. Treatments sharing letters of the same case had non-significant differences ($p > 0.05$). Error bars show 1 standard deviation ($N = 3$)

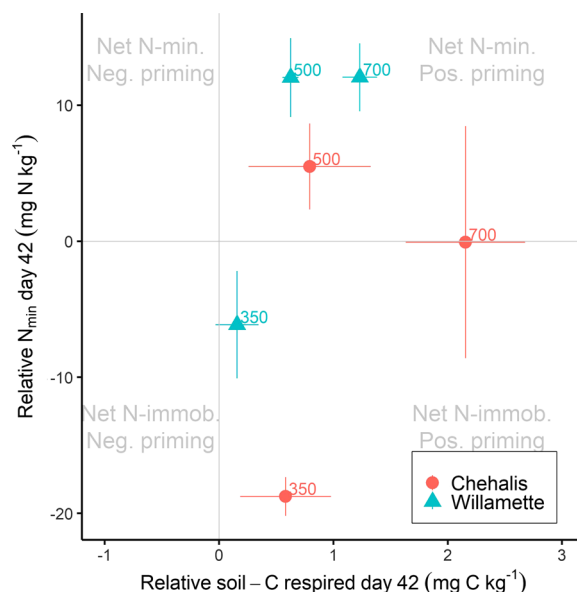


Fig. 3 Net soil N-mineralization and cumulative soil-C respired on day 42 in barley biochar-amended soils, with quantities from unamended soils subtracted. Error bars are standard errors. Quadrants indicate whether biochar amendment increased or decreased N-mineralization and soil-C respiration relative to unamended soils

the upper right quadrat—priming soil C and mineralizing soil N.

Characteristics of the biochars and biochar + soil systems were evaluated as potential explanatory variables. Consistent with expectation, the C:N ratio of the biochar + soil system (Fig. 4a), and the C:N ratio of the biochars themselves (Fig. 4b) did not correlate with net N-mineralized by day 42. However, in support of hypothesis 1, a negative correlation was found between net N-mineralization and the C_{\min} :total N ratio (slope p -value = 0.009, $R^2 = 0.96$ for a model with a single slope for the two soils). We note, however, the limited distribution of the data composing this regression, given the similarity between the 500 and 700 °C biochars. Because total-N quantities were similar for the three barley biochars (Table 1), the variation in soil N-mineralization was also explained well by C_{\min} content alone, with almost identical statistical results. Contrary to hypothesis 2, no correlation

was found between the C:N ratio of biochar water extracts and soil N-mineralization (Fig. 4d).

3.2 Experiment 2: expanding to more biochar types

In the second experiment, the barley plant biochars and seven additional biochar types were incubated in Willamette soil (1.7% total C). The response of the barley biochars was compared in both experiments to assess repeatability. Soil N-mineralization, which was measured on day 14 in both experiments, was about 25% lower in the first experiment than in the second (data not shown), possibly related to a fresh collection of Willamette soil made in spring 2019 compared to fall 2018. Similar to experiment 1, the barley biochars in experiment 2 exhibited increased soil N-mineralization with increasing pyrolysis temperatures (Fig. 5).

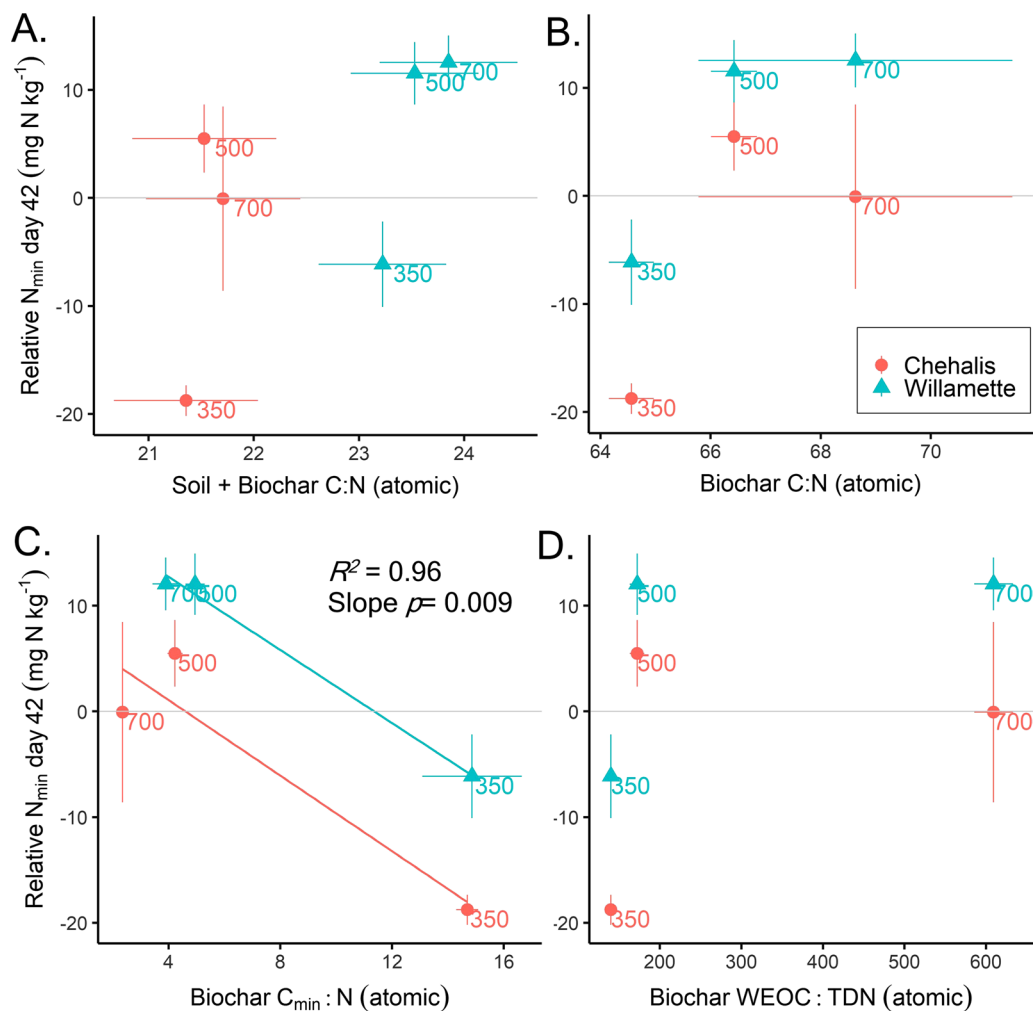


Fig. 4 Correlations between relative net mineralized-N after 42 days (relative=amended minus unamended soil) and characteristics of barley biochar and biochar + soil system, including **A** biochar + soil C to N ratio, **B** biochar only C to N ratio, **C** biochar cumulative

respired-C in 42 days to N ratio, and **D** biochar water-extractable organic C to total dissolved N ratio. Biochars were pyrolyzed at 350, 500, and 700 °C. Error bars show standard deviations ($N=3$)

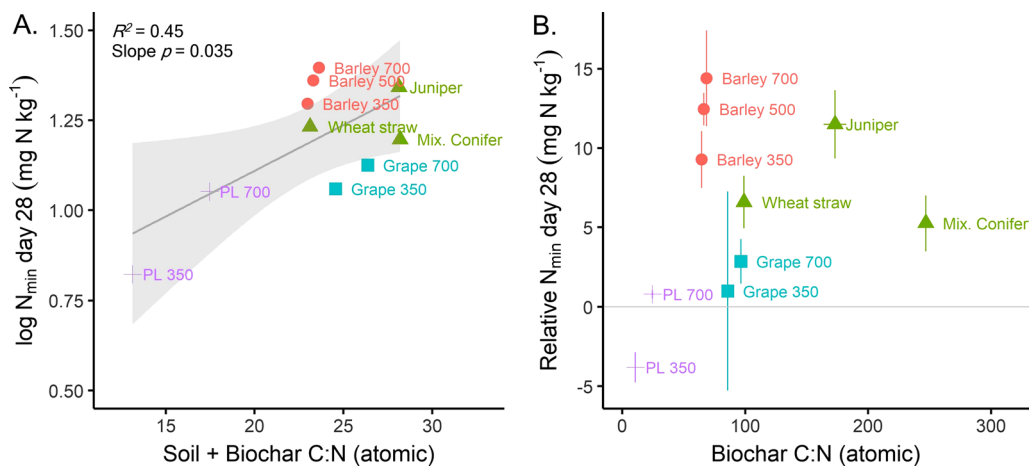


Fig. 5 N-mineralization in 28 days in Willamette soil versus C:N ratio of **A** the soil + biochar system, and **B** biochars alone. In **A** log-transformed N-mineralization had a significant correlation with soil + biochar C:N. N-mineralization also increased with pyroly-

sis temperature for individual feedstocks. In **B**, N-mineralization is shown as the difference between amended and unamended soil for comparison to Fig. 6. Error bars are standard deviations (too small to be visible in **A**, $N = 3$)

All but one of the biochars had a neutral or stimulatory effect on 28-day soil N-mineralization relative to unamended soil (Fig. 5b). Only the 350 °C poultry litter biochar reduced 28-day N-mineralization, but it stimulated high 14-day net N-mineralization ($37.8 \pm 24.3 \text{ mg N kg}^{-1}$) followed by immobilization and low net N-mineralization on day 28 ($6.65 \pm 0.94 \text{ mg N kg}^{-1}$). The rapid N-mineralization of the 350 °C poultry litter biochar was likely due to its very high N content, primarily in the form of NH_4^+ and organic-N (Table 1). All the other biochars had an approximate doubling of net N-mineralization from day 14 to day 28 (see supplemental materials, Fig. S2).

Soil N-mineralization in 28 days correlated positively with the C:N ratio of soil + biochar system (Fig. 5a), but not with the C:N ratio of biochar alone (Fig. 5b). When evaluated with respect to the C_{\min} content of the biochars, the larger set of biochars provided evidence to support the hypothesis that soil N-mineralization correlates negatively with biochar C_{\min} within individual feedstock types, but not across different feedstocks (Fig. 6).

Overall, soil N-mineralization increased with higher pyrolysis temperatures while C_{\min} tended to decrease. Increases in soil N-mineralization with biochar pyrolysis temperature were observed for the barley plant, grape wood, and poultry litter biochar types, although variances were high for the 350 °C grape wood biochar. Simultaneously, C_{\min} content as quantified by all three measures— KMnO_4 -oxidation, H:C ratio, and WEOC—generally decreased with increasing pyrolysis temperature (Fig. 6a-c). However, the three measures of C_{\min} were not entirely consistent with each other (see Fig. S1). A notable exception to C_{\min} decreasing with pyrolysis temperature was that the poultry litter biochar had more KMnO_4 -oxidizable C at 700 °C than at 350 °C

(Fig. 6a). The decline in C_{\min} with pyrolysis temperature was most consistent for the H:C measure (Fig. 6b).

Relationships between biochar C_{\min} and soil N-mineralization were weaker across feedstock types. A regression model of soil N-mineralization as a function of H:C and feedstock type showed a significant correlation when only pyrolysis biochars were considered (Fig. 6b, $p = 0.013$ for effect of H:C, $R^2 = 0.98$) but a weaker correlation when the combustion biochars were included ($p = 0.11$ for effect of H:C, $R^2 = 0.84$). The C:N ratio of biochar water extracts had no consistent relationship with soil N-mineralization, either within individual feedstocks or across the different feedstocks (Fig. 6d).

PCA was used to explore additional biochar characteristics apart from C_{\min} and dissolved C:N ratio that were associated with mineralization of soil N (Fig. 7). Initially, all three measures of biochar C_{\min} were included in the analysis, but KMnO_4 -oxidizable C had low loadings to the first two axes and was subsequently excluded to reduce ordination complexity. The first two axes of the PCA model explained 74% of the variance in the 9 biochar characteristics included in the model. Overall, the first principal component distinguished biochar feedstocks and the second principal component distinguished pyrolysis temperatures (Fig. 7a). The combustion biochars also segregated from pyrolysis biochars on component 2. The variables with the largest loadings in component 1 were 28-day N-mineralization and biochar C:N, which correlated positively with each other (Fig. 7b), while inorganic C content, which increased with pyrolysis temperature (Table 1), had the largest loading in component 2. Soil N-mineralization was negatively correlated with extractable-P, -K, and -Mg, and TDN. This reflected low N-mineralization rates from the three most

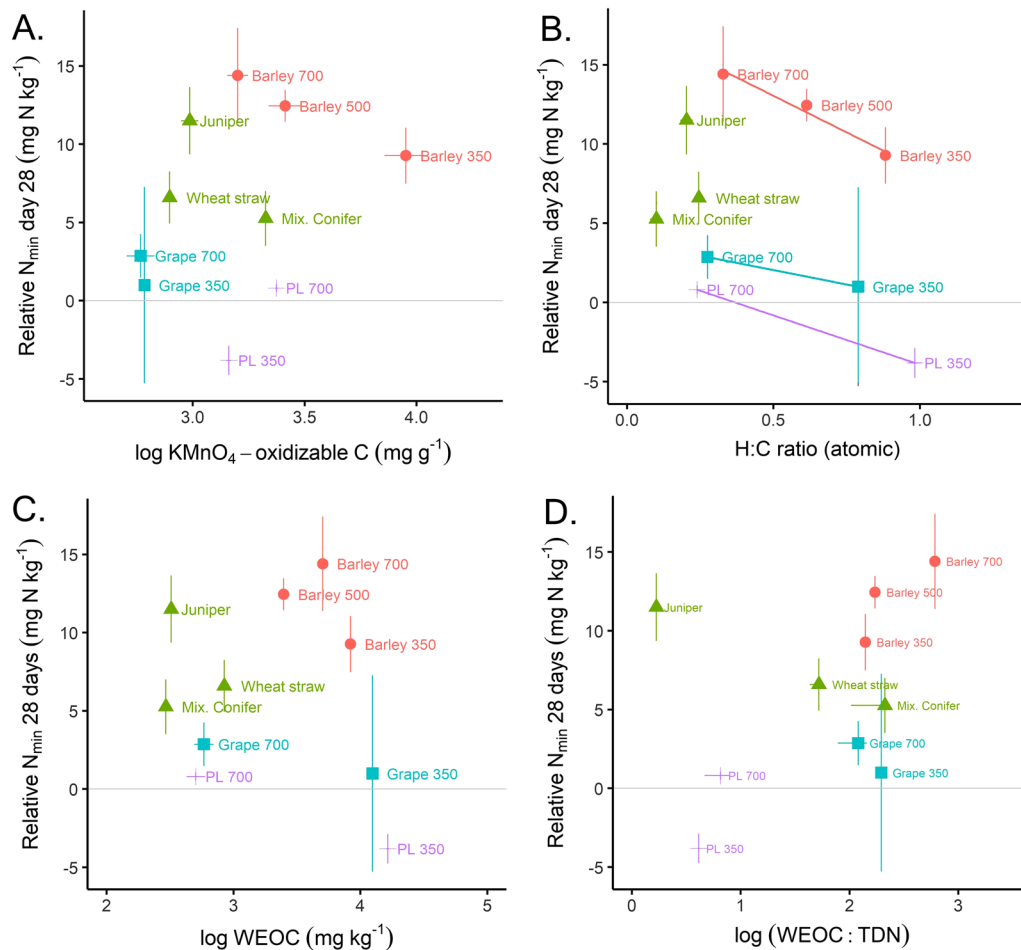


Fig. 6 Relative net N-mineralization in 28 days in Willamette soil (relative = amended minus unamended soil), versus biochar mineralizable-C characteristics. Biochar mineralizable component was characterized by **A** KMnO_4 -oxidizable C content, **B** atomic H:C ratio, **C**

water-extractable organic carbon content, and **D** the ratio of water-extractable organic carbon to total dissolved N. Error bars are standard deviations ($N=3$)

nutrient dense biochars—the 350 °C grape wood and the two poultry litter biochars. H:C and WEOC, which were hypothesized to correlate negatively with soil N-mineralization, were orthogonal to N-mineralization in the PCA analysis. The multivariate analysis thus demonstrated possible unique responses of the most nutrient-dense biochars that were simultaneously higher in soluble N, and extractable P, Mg, and K.

4 Discussion

4.1 Generalizing soil impacts from biochar characteristics

A goal of this study was to evaluate whether biochar impacts on soil DIN content could be predicted from readily measured biochar C- and N- pools. The results suggested that

generalizations of soil-N impacts were only predictable within a biochar feedstock. The hypothesis that soil N-mineralization correlates negatively with biochar C_{\min} was only somewhat supported for individual feedstocks produced at a range of temperatures (Figs. 4c and 6b) and was not supported across feedstocks and when pyrolysis and combustion chars were aggregated (Fig. 6b). Consistent with previous studies, we found greater variation in soil-N response across biochar feedstocks than across production temperatures (Rajkovich et al. 2012; Maaz et al. 2021).

Our results highlighted the limited utility of biochar total C:N ratios in predicting soil responses. In both experiments, soil N-mineralization tended to increase with the C:N ratio of soil + biochar system (Figs. 4a and 5a). This result directly contrasts with traditional models of decomposition and evidence from diverse ecosystems suggesting that increasing soil C:N ratios should reduce N-mineralization rates (Booth et al. 2005). Additionally, across a large range of biochar

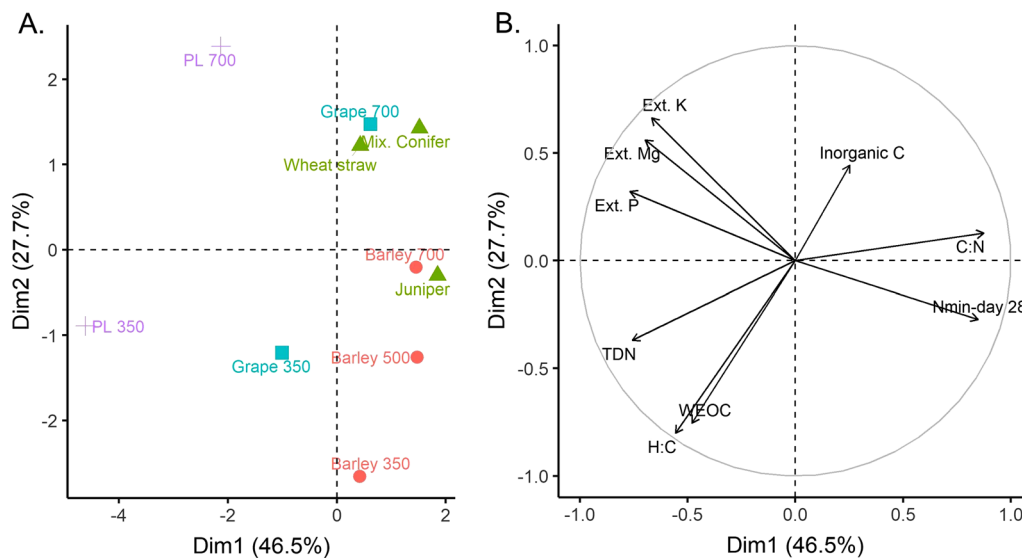


Fig. 7 Principal component analysis (PCA) ordination of selected biochar characteristics, showing **A** biochar positions and **B** loadings of biochar characteristics

C:N ratios ranging from approximately 10 to 250, we found soil N-mineralization had no relationship with biochar C:N (Fig. 5b). This agrees with a large meta-analysis by Gao et al. (2019) that showed inconsistent impacts of C:N on soil N-NO₃⁻ and N-NH₄⁺.

However, a limitation of our study was the lack of diverse measurements on the C:N ratio of the biologically-active component of the biochars. Biochar C_{min} content was characterized with four methods (see supplemental Fig. S1 for comparisons of C_{min} measures for the barley biochar temperature series). However, the N content of the mineralizable component was estimated only from water-extractable organic matter, and C data suggested that water extracts were only a small portion of the total biologically-active pool. C_{min} estimates from isotopic partitioning of respired CO₂ (Fig. 1a) were approximately 10 times greater than WEOC (Table 1), suggesting a considerable portion of biologically-available C and N were not contained in the water-extractable pool. Additionally, the lack of correlation between WEOC:TDN and soil N-mineralization suggested that biochar water extracts were insufficient to describe biologically-available N (Figs. 4d and 6d).

Our findings agree with others who have shown that generalizations of biochar impacts on soil N are difficult to make from simple biochar characterizations. For example, volatile matter (VM) content is an easily measured property that corresponds with a biochar’s degree of thermal alteration and C_{min} content (Zimmerman 2010; Enders et al. 2012). However, the composition of the VM component has been shown to be chemically heterogeneous (Spokas 2010; Spokas et al. 2011; Maaz et al. 2021), with variable impacts on soil microbial activity and N-transformations. Molecular structure

measurements can help to describe the non-homogenous composition of pyrogenic organic matter (Heckman et al. 2017; Torres-Rojas et al. 2020) and will likely be useful in future work to generalize soil N-impacts from biochar characteristics. For instance, the PCA analysis (Fig. 7) showed that extractable nutrients had a *negative* correlation with soil N-mineralization. Molecular structure measurements may help show why the most nutrient-dense biochars had only a small impact on N-mineralization.

4.2 Linkages between soil C- and N-mineralization

A surprising result from this study was that all the biochars stimulated N-mineralization relative to unamended soil (Figs. 5b and 6). Only the low-temperature poultry litter biochar yielded less DIN than unamended soil after 28 days, but this was due to N-immobilization following a large pulse of N-mineralization earlier in the incubation (Fig. S2). Based on prior studies we expected most of the biochars to have no impact on DIN (Zheng et al. 2012) or to reduce DIN relative to unamended soil, with only high-N biochars (e.g., from manure) stimulating increases in DIN (Nguyen et al. 2017; Gao et al. 2019). Our results did not support this expectation, as the high-N (as measured by TDN) 700 °C poultry litter biochar yielded low quantities of DIN in soil incubations, while the low-N 700 °C barley biochar yielded the highest quantities of DIN (Figs. 5 and 6).

A second surprising result was that all three barley biochars in experiment 1 stimulated positive priming (Fig. 3). We had expected at least some of the biochars to cause negative priming, i.e., decreases in soil-C respiration. Although both positive and negative priming have been reported from

biochar additions (Zimmerman 2010; Stewart et al. 2013; Whitman et al. 2014), a meta-analysis of 21 studies showed that incubations less than 6 months in duration generally suppressed soil respiration, and furthermore that negative priming was more common than positive priming in soils with clay contents between 10 and 40% (Wang et al. 2016), such as the Willamette and Chehalis soil types. Viewed in terms of the binary positive or negative impacts of biochar on soil N- and C-mineralization illustrated in Fig. 3, we expected at least some of the barley biochars to fall on the left side, corresponding with negative priming. Additionally, we were surprised that the 500 °C and 700 °C biochars both stimulated more positive priming than the 350 °C biochar, despite their lower C_{\min} content. Several studies have previously reported the reverse trend, with high-temperature biochars having less impact or reducing soil respiration compared to low temperature biochars (Cross and Sohi 2011; Ameloot et al. 2015; Wang et al. 2016).

The finding that biochars with more microbially-resistant biochemistry stimulated more soil decomposition is consistent, however, with previous isotopic studies that evaluated partitioning between soil and residue decomposition. Studying five uncharred crop residues, Stewart et al. (2015) demonstrated that a direct trade-off occurred between respiration of soil-derived C and residue-derived C in incubations. Residues that decayed the least produced the most soil-derived C, and vice versa. Because residue- and soil-respired C balanced each other, total C respired over the incubation was unrelated to residue quality. Whitman et al. (2014) also showed a trade-off between CO_2 respired from biochar and soil in the first 48 h of incubations. High rates of respired soil-C corresponded with low rates of biochar-C respiration, in a highly significant linear relationship.

Our finding of more soil N-mineralization stimulated by high-temperature barley biochars was surprising given their low N-content and high C:N ratios, but was consistent with the stimulatory effect these biochars had on soil C-mineralization (Fig. 3). We also noted a difference between the two soil types, with biochar stimulating more C- and N-mineralization from the low-C Willamette soil (Figs. 1b and 2). Whitman et al. (2014) also found a difference in the responses of two soils, with stronger biochar priming effects in soils with less mineralizable-C. This stronger priming effect in the Willamette soil, as well as its higher initial C:N ratio which indicated less N-limitation (Table 1), likely explained the greater N-mineralization in the Willamette soil. These results showed that considering both biochar and soil characteristics together is important to determine amendment impacts on plant-available N.

Although uncommon, reports of biochar stimulating N-mineralization are not without precedent. For instance, additions of wood biochars to an organically-farmed soil in coastal Washington State increased potentially-mineralizable

N levels (Gao et al. 2016), despite the fact that the biochars were poor sources of N.

4.3 Methodologies for biochar C_{\min} and soil N-mineralization

There are a large number of methods for characterizing biochar C_{\min} and soil N-mineralization, providing a spectrum from expediency on the one hand to mechanistic detail on the other hand. The three non-isotopic measures used to evaluate biochar C_{\min} —H:C ratio, $KMnO_4$ -oxidizable C, and WEOC—can be viewed as proxies for isotopic partitioning methods, which are preferred as a direct measure of biochar C mineralization (Budai et al. 2013; Leng et al. 2019). For the barley biochars, we found H:C ratio and $KMnO_4$ -oxidizable C content corresponded well with isotopic measures of C_{\min} , but that WEOC had a less linear relationship (Fig. S1). We also noted that both $KMnO_4$ -oxidizable C and WEOC yielded about 10% of C_{\min} detected by isotopic measurements (Table 1 and Fig. 1b), thus providing quasi-quantitative measures of C_{\min} . The H:C ratio has been recommended as a community standard for estimating biochar stability, and has been shown to correspond well with degree of aromaticity and biochar residence times from long-term incubations (Lehmann et al. 2015; Xiao et al. 2016). H:C ratio was also the C_{\min} measure that corresponded best with soil N-mineralization in experiment 2. Although H:C ratio does not provide a direct measure of C_{\min} content, we agree with its use as a non-isotopic assessment of biochar C_{\min} . Although we found good agreement between $KMnO_4$ -oxidizable C and respired biochar-C for the barley biochars, it was critiqued by Calvelo Pereira et al. (2011) who found it did not consistently agree with other thermal and oxidative measures of C_{\min} . Additionally, $KMnO_4$ -oxidizable C has been criticized for oxidizing many classes of compounds indiscriminately without reflecting microbial preference (Tirol-Padre and Ladha 2004).

Many laboratory methods are also used for characterizing soil N-mineralization, with common incubation times ranging from one to 30 weeks (Drinkwater et al. 1996; Sharifi et al. 2007). We used a 28-day aerobic incubation in experiment 2 and focused on day 42 for hypothesis testing in experiment 1, because our interest was to establish an approach for screening short-term impacts of many biochars. However, as described by Sharifi et al. (2007), different pools of mineralizable-N are recognized over different periods of incubation. Over the longer term (> 1 year), Nguyen et al. (2017) showed less impact of biochar on soil $N-NH_4^+$ but larger reductions in $N-NO_3^-$ compared to short-term studies. Characterizing a wide range of biochars with inherently different mineralizable fractions in a short-term incubation likely misses important aspects, and assessing long-term

outcomes is also important for completely characterizing biochar impacts on soil-N.

5 Conclusions

This study provided initial steps towards evaluating biochar characteristics that may be useful for predicting negative impacts on plant-available N. We showed that biochar C:N ratio was a poor predictor of soil N-mineralization, but that for a given feedstock, biochar C_{\min} content had a negative relationship with soil N-mineralization. Nevertheless, biochar C_{\min} was not related to soil N-mineralization across different feedstocks and biochar production methods, suggesting that C_{\min} is a chemically heterogeneous pool with variable impacts on soil plant-available N content.

Perhaps one of the most interesting findings was that a diverse number of biochars all stimulated N-mineralization in the two soils that were studied. Based on prior studies, we expected biochar to have an overall neutral or suppressive impact on N-mineralization. However, our study indicated that despite having high C:N ratios and low N contents, biochars “unlocked” plant-available N through positive priming of soil C. These observations may be related to the fact that the two soils studied both had relatively high C contents, which readily supported the simultaneous decomposition of soil-C along with the added biochar-C. Still more surprising was that for a given feedstock, plant-available N was greater in response to high-temperature biochars that were more resistant to microbial decomposition than low-temperature biochars. These results add complexity to the range of soil-N responses that can be expected in response to biochar amendment. Because of the lack of easily measured biochar characteristics to predict soil-N responses, biochar users should monitor soil-N impacts of biochar amendment to adaptively-manage soil fertility.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42773-022-00137-2>.

Acknowledgements L. Pruessner, M. Cruse, J. Vasen, P. Mahato, and K. Sugimoto of USDA-ARS provided laboratory assistance. P. Hayes and D. Bryla provided biochar feedstocks. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Author contributions The study was conceptualized and designed by CLP, KMT, TW, MG-J, and CES. Material preparation was performed by MG, DW, JN, and KM. Data collection was performed by CLP, KM, CW, MG-J, TW, and CES. Analyses were performed and the first draft of the manuscript was written by CLP. All authors commented on previous versions of the manuscript and approved the final manuscript. Funding acquisition and supervision were provided by KMT.

Funding This work was supported by the USDA Agricultural Research Service projects in the laboratories of K.M.T (2072-12620-001), C.L.P. (5030-12000-015), C.E.S (3012-11120-001), J.M.N. (6082-12630-001), and M.A.G. (3060-43440-016), and a Grant from the Institute for Working Forest Landscapes at Oregon State University.

Availability of data and materials The datasets analyzed during the current study are available from the U.S.D.A. AgData Commons at the following URL: <https://doi.org/10.15482/USDA.ADC/1523372>.

Code availability The code used for statistical analyses and generating figures are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Ågren GI, Hyvönen R, Berglund SL, Hobbie SE (2013) Estimating the critical N: C from litter decomposition data and its relation to soil organic matter stoichiometry. *Soil Biol Biochem* 67:312–318. <https://doi.org/10.1016/j.soilbio.2013.09.010>
- Ameloot N, Sleutel S, Das KC et al (2015) Biochar amendment to soils with contrasting organic matter level: effects on N mineralization and biological soil properties. *GCB Bioenergy* 7:135–144. <https://doi.org/10.1111/gcbb.12119>
- Andrews N, Foster J (2007) Organic fertilizer calculator: a tool for comparing the cost, nutrient value, and nitrogen availability of organic materials. Oregon State University Extension Service
- Aulakh MS, Walters DT, Doran JW et al (1991) Crop residue type and placement effects on denitrification and mineralization. *Soil Sci Soc Am J* 55:1020–1025. <https://doi.org/10.2136/sssaj1991.03615995005500040022x>
- Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Org Geochem* 33:1093–1109. [https://doi.org/10.1016/S0146-6380\(02\)00062-1](https://doi.org/10.1016/S0146-6380(02)00062-1)
- Bonomi G, Ippolito F, Cesarano G et al (2017) Biochar as plant growth promoter: better off alone or mixed with organic amendments? *Front Plant Sci* 8:1570. <https://doi.org/10.3389/fpls.2017.01570>
- Booth MS, Stark JM, Rastetter E (2005) Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecol Monogr* 75:139–157. <https://doi.org/10.1890/04-0988>

- Budai A, Zimmerman AR, Cowie AL, et al (2013) Biochar carbon stability test method: an assessment of methods to determine biochar carbon stability. International Biochar Initiative
- Budai A, Rasse DP, Lagomarsino A et al (2016) Biochar persistence, priming and microbial responses to pyrolysis temperature series. *Biol Fertil Soils* 52:749–761. <https://doi.org/10.1007/s00374-016-1116-6>
- Calvelo Pereira R, Kaal J, Camps Arbestain M et al (2011) Contribution to characterisation of biochar to estimate the labile fraction of carbon. *Org Geochem* 42:1331–1342. <https://doi.org/10.1016/j.orggeochem.2011.09.002>
- Calvelo Pereira R, Camps-Arbestain M, Wang T, Enders A (2017) Inorganic carbon. In: Singh B, Arbestain MC, Lehmann J (eds) *Biochar: a guide to analytical methods*. CRC Press, Taylor & Francis Group, Boca Raton, pp 51–63
- Camps-Arbestain M, Amonette JE, Singh B et al (2015) A biochar classification system and associated test methods. In: Lehmann J, Joseph S (eds) *Biochar for environmental management: science, technology and implementation*, 2nd edn. Sterling, London, p 28
- Cantrell KB, Martin JH (2012) Stochastic state-space temperature regulation of biochar production. Part I: theoretical development. *J Sci Food Agric* 92:481–489. <https://doi.org/10.1002/jsfa.4618>
- Chinu K, Marjo CE, Joseph S, Singh B (2017) Dissolved carbon and LC-OCD of biochar. In: Singh B, Arbestain MC, Lehmann J (eds) *Biochar: a guide to analytical methods*. CRC Press, Taylor & Francis Group, Boca Raton London, pp 64–73
- Clough TJ, Condron LM (2010) Biochar and the nitrogen cycle: introduction. *J Environ Qual* 39:1218–1223. <https://doi.org/10.2134/jeq2010.0204>
- Cross A, Sohli SP (2011) The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biol Biochem* 43:2127–2134. <https://doi.org/10.1016/j.soilbio.2011.06.016>
- Deenik JL, McClellan T, Uehara G et al (2010) Charcoal volatile matter content influences plant growth and soil nitrogen transformations. *Soil Sci Soc Am J* 74:1259–1270. <https://doi.org/10.2136/sssaj2009.0115>
- Doane TA, Horwath WR (2003) Spectrophotometric determination of nitrate with a single reagent. *Anal Lett* 36:2713–2722. <https://doi.org/10.1081/AL-120024647>
- Drinkwater LE, Cambardella CA, Reeder JD, Rice CW (1996) Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen. In: Doran JW, Jones AJ (eds) *SSSA special publications*. Soil Science Society of America, Madison, pp 217–229
- Ducey TF, Ippolito JA, Cantrell KB et al (2013) Addition of activated switchgrass biochar to an aridic subsoil increases microbial nitrogen cycling gene abundances. *Appl Soil Ecol* 65:65–72. <https://doi.org/10.1016/j.apsoil.2013.01.006>
- Enders A, Hanley K, Whitman T et al (2012) Characterization of biochars to evaluate recalcitrance and agronomic performance. *Bioresour Technol* 114:644–653. <https://doi.org/10.1016/j.biortech.2012.03.022>
- Gao S, Hoffman-Krull K, Bidwell AL, DeLuca TH (2016) Locally produced wood biochar increases nutrient retention and availability in agricultural soils of the San Juan Islands, USA. *Agr Ecosyst Environ* 233:43–54. <https://doi.org/10.1016/j.agee.2016.08.028>
- Gao S, DeLuca TH, Cleveland CC (2019) Biochar additions alter phosphorus and nitrogen availability in agricultural ecosystems: a meta-analysis. *Sci Total Environ* 654:463–472. <https://doi.org/10.1016/j.scitotenv.2018.11.124>
- Heckman K, Torres D, Swanston C, Lehmann J (2017) Carbon and nitrogen molecular composition of soil organic matter fractions resistant to oxidation. *Soil Res* 55:809. <https://doi.org/10.1071/SR16182>
- Husson F, Josse J, Le S, Mazet J (2020) “FactoMineR”: multivariate exploratory data analysis and data mining. Version 2.4 URL <http://factominer.free.fr>
- Jeffery S, Abalos D, Spokas KA, Verheijen FGA (2015) Biochar effects on crop yield. In: Lehmann J, Joseph S (eds) *Biochar for environmental management: science, technology and implementation*. Sterling, London, pp 301–325
- Kassambara A, Mundt F (2020) “factoextra”: Extract and Visualize the Results of Multivariate Data Analyses. Version 1.0.7 URL <http://www.sthda.com/english/rpkgs/factoextra>
- Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic molecular structure of plant biomass-derived black carbon (Biochar). *Environ Sci Technol* 44:1247–1253. <https://doi.org/10.1021/es9031419>
- Kuzyakov Y, Subbotina I, Chen H et al (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by ¹⁴C labeling. *Soil Biol Biochem* 41:210–219. <https://doi.org/10.1016/j.soilbio.2008.10.016>
- Lehmann J, Abiven S, Kleber M et al (2015) Persistence of biochar in soil. In: Lehmann J, Joseph S (eds) *Biochar for environmental management: science, technology and implementation*, 2nd edn. Routledge, Taylor & Francis Group, London
- Leng L, Huang H, Li H et al (2019) Biochar stability assessment methods: a review. *Sci Total Environ* 647:210–222. <https://doi.org/10.1016/j.scitotenv.2018.07.402>
- Lifschitz CH, Grusak MA, Butte NF (2002) Carbohydrate digestion in humans from a β -glucan-enriched barley is reduced. *J Nutr* 132:2593–2596. <https://doi.org/10.1093/jn/132.9.2593>
- Liu Z, Dugan B, Masiello CA, Gonnermann HM (2017) Biochar particle size, shape, and porosity act together to influence soil water properties. *PLoS ONE* 12:1–19. <https://doi.org/10.1371/journal.pone.0179079>
- Liu Q, Zhang Y, Liu B et al (2018) How does biochar influence soil N cycle? A meta-analysis. *Plant Soil* 426:211–225. <https://doi.org/10.1007/s11104-018-3619-4>
- Maaz TM, Hockaday WC, Deenik JL (2021) Biochar volatile matter and feedstock effects on soil nitrogen mineralization and soil fungal colonization. *Sustainability* 13:2018. <https://doi.org/10.3390/su13042018>
- Manzoni S (2017) Flexible carbon-use efficiency across litter types and during decomposition partly compensates nutrient imbalances—results from analytical stoichiometric models. *Front Microbiol* 8:661. <https://doi.org/10.3389/fmicb.2017.00661>
- Mia S, Singh B, Dijkstra FA (2017) Aged biochar affects gross nitrogen mineralization and recovery: a 15N study in two contrasting soils. *GCB Bioenergy* 9:1196–1206. <https://doi.org/10.1111/gcbb.12430>
- Nguyen TTN, Xu C-Y, Tahmasbian I et al (2017) Effects of biochar on soil available inorganic nitrogen: a review and meta-analysis. *Geoderma* 288:79–96. <https://doi.org/10.1016/j.geoderma.2016.11.004>
- Phillips CL, Light SE, Gollany HT et al (2020a) Can biochar conserve water in Oregon agricultural soils? *Soil Till Res* 198:104525. <https://doi.org/10.1016/j.still.2019.104525>
- Phillips CL, Meyer KM, Trippe KM (2020b) Is biochar applied as surface mulch beneficial for grassland restoration? *Geoderma* 375:114457. <https://doi.org/10.1016/j.geoderma.2020.114457>
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rajan SSS, Brown MW, Boyes MK, Upsdell MP (1992) Extractable phosphorus to predict agronomic effectiveness of ground and unground phosphate rocks. *Fert Res* 32:291–302. <https://doi.org/10.1007/BF01050366>
- Rajkovich S, Enders A, Hanley K et al (2012) Corn growth and nitrogen nutrition after additions of biochars with varying properties

- to a temperate soil. *Biol Fertil Soils* 48:271–284. <https://doi.org/10.1007/s00374-011-0624-7>
- Rayment GE, Lyons DJ (2011) *Soil chemical methods: Australasia*. CSIRO Publishing, Collingwood
- Rhine ED, Mulvaney RL, Pratt EJ, Sims GK (1998) Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Sci Soc Am J* 62:473. <https://doi.org/10.2136/sssaj1998.03615995006200020026x>
- Sharifi M, Zebarth BJ, Burton DL et al (2007) Evaluation of some indices of potentially mineralizable nitrogen in soil. *Soil Sci Soc Am J* 71:1233–1239. <https://doi.org/10.2136/sssaj2006.0265>
- Soil Survey Staff U-N (2006) *Official Soil Series Descriptions* [Online]. United States Department of Agriculture, Lincoln, NE
- Spokas KA (2010) Review of the stability of biochar in soils: predictability of O:C molar ratios. *Carbon Manag* 1:289–303. <https://doi.org/10.4155/cmt.10.32>
- Spokas KA, Novak JM, Stewart CE et al (2011) Qualitative analysis of volatile organic compounds on biochar. *Chemosphere* 85:869–882. <https://doi.org/10.1016/j.chemosphere.2011.06.108>
- Spokas KA, Cantrell KB, Novak JM et al (2012) Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *J Environ Qual* 41:973–989. <https://doi.org/10.2134/jeq2011.0069>
- Stewart CE, Zheng J, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar mitigates green-house gas emissions and increases carbon sequestration in temperate soils. *GCB Bioenergy* 5:153–164. <https://doi.org/10.1111/gcbb.12001>
- Stewart CE, Moturi P, Follett RF, Halvorson AD (2015) Lignin biochemistry and soil N determine crop residue decomposition and soil priming. *Biogeochemistry* 124:335–351. <https://doi.org/10.1007/s10533-015-0101-8>
- Tirol-Padre A, Ladha JK (2004) Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. *Soil Sci Soc Am J* 68:969–978. <https://doi.org/10.2136/sssaj2004.9690>
- Torres-Rojas D, Hestrin R, Solomon D et al (2020) Nitrogen speciation and transformations in fire-derived organic matter. *Geochim Cosmochim Acta* 276:170–185. <https://doi.org/10.1016/j.gca.2020.02.034>
- Trinsoutrot I, Recous S, Bentz B et al (2000) Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Sci Soc Am J* 64:918–926. <https://doi.org/10.2136/sssaj2000.643918x>
- Vigil MF, Kissel DE (1991) Equations for estimating the amount of nitrogen mineralized from crop residues. *Soil Sci Soc Am J* 55:757–761. <https://doi.org/10.2136/sssaj1991.03615995005500030020x>
- Wang T, Camps-Arbestain M, Hedley M, Bishop P (2012) Predicting phosphorus bioavailability from high-ash biochars. *Plant Soil* 357:173–187. <https://doi.org/10.1007/s11104-012-1131-9>
- Wang J, Xiong Z, Kuzyakov Y (2016) Biochar stability in soil: meta-analysis of decomposition and priming effects. *GCB Bioenergy* 8:512–523. <https://doi.org/10.1111/gcbb.12266>
- Weil R, Islam K, Stine MA et al (2003) Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. *Am J Altern Agric* 18:3–17
- Whitman T, Zhu Z, Lehmann J (2014) Carbon mineralizability determines interactive effects on mineralization of pyrogenic organic matter and soil organic carbon. *Environ Sci Technol* 48:13727–13734. <https://doi.org/10.1021/es503331y>
- Woolf D, Amonette JE, Street-Perrott FA et al (2010) Sustainable biochar to mitigate global climate change. *Nat Commun* 1:56. <https://doi.org/10.1038/ncomms1053>
- Xiao X, Chen Z, Chen B (2016) H/C atomic ratio as a smart linkage between pyrolytic temperatures, aromatic clusters and sorption properties of biochars derived from diverse precursory materials. *Sci Rep* 6:22644. <https://doi.org/10.1038/srep22644>
- Zhang A, Liu Y, Pan G et al (2012) Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant Soil* 351:263–275. <https://doi.org/10.1007/s11104-011-0957-x>
- Zheng J, Stewart CE, Cotrufo MF (2012) Biochar and nitrogen fertilizer alters soil nitrogen dynamics and greenhouse gas fluxes from two temperate soils. *J Environ Qual* 41:1361–1370. <https://doi.org/10.2134/jeq2012.0019>
- Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (Biochar). *Environ Sci Technol* 44:1295–1301. <https://doi.org/10.1021/es903140c>