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Biochar alters nitrogen and phosphorus dynamics in a western rangeland ecosystem

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ABSTRACT

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Application of biochar to soils has been proposed as a novel approach to managing wood residuals, enhancing soil carbon (C) storage and improving soil fertility; however, the majority of biochar studies have been conducted in agricultural systems that rely on tillage and nutrient inputs associated with annual cropping schemes. Few studies have evaluated the influence of biochar on soil processes in semi-natural rangeland ecosystems that feature more complex plant communities, lack deep soil disturbance, and have relatively few external nutrient inputs. In August 2018, biochar produced using wood waste from a lumber mill in Columbia Falls, MT, USA was applied to surface soils in replicated plots at an experimental ranch in western Montana to test the impact of biochar on soil C storage and nutrient management. A series of soil biochemical properties including total soil C and nitrogen (N), microbial N functional genes, available phosphorus (P) and the net accumulation of nutrients below surface soil layer were evaluated over a one-year period following biochar addition with or without a poultry litter based organic fertilizer. Biochar used alone slightly reduced soil NH4, significantly increased soil nitrification potential, increased the relative abundance of the bacterial amoA gene, and increased the soil nitrate (NO_3^-) pool size, while having no net effect on soil inorganic N accumulation below surface soil. By contrast, biochar charged with poultry litter (termed "charged biochar") had no effect on NH⁴₄ availability, but had a positive effect on amoA abundance. Charged biochar significantly reduced NH⁴₄ accumulation below 25 cm depth compared to poultry litter alone. Biochar additions led to a shift towards a more fungal dominated community and a general increase in P availability. However, biochar alone also contributed to a greater amount of soluble P collected below surface soil, an effect slightly attenuated when biochar was applied with poultry litter. Soil pH increased from 5.7 to 6.9 in response to biochar addition and was one of the dominant factors governing the observed changes in soil processes. Charged biochar helped retain soil nutrients and promoted soil C storage in this semi-natural rangeland system over one growing season. Changes in these soil pools and fluxes may influence various trophic groups affecting ecosystem functioning over time.

1. Introduction

Biochar production and application to soils has been promoted as an effective way of recycling biomass while benefiting soil carbon (C) sequestration, soil moisture and nutrient retention, and alleviating nutrient leaching (DeLuca and Gao, 2019; Gao et al., 2017). To date, however, the majority of biochar studies have focused on row crop agricultural systems that are typically associated with relatively low species diversity and high dependence upon external nutrient inputs (Gao et al., 2019; Nair et al., 2017). By contrast, relatively few studies have investigated the functions of wood biochar on soil processes in semi-natural rangeland settings that feature more complex, perennial

plant communities, relatively few external nutrient inputs, and experience limited disturbance other than physical (e.g freeze-thaw effects) or biological pedoturbation in place of annual practices of soil tillage and crop harvest (Shamin, 2018; van de Voorde et al., 2014).

Previous studies have suggested that biochar amendment of soil in natural systems with high biodiversity could affect the competitive hierarchy of plant species, which, over time, may lead to ecosystem-scale species turnover (van de Voorde et al., 2014). The hypothesized mechanisms for this community composition shift with the presence of biochar or pyrogenic C is a shift in soil nutrient cycling and plant-soil interactions where biochar could affect seed germination and plant establishment by adsorbing soil allelochemicals (DeLuca and Sala, 2006;

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Gundale and DeLuca, 2006; MacKenzie et al., 2006), altering rhizosphere environment that could favor specific plant species while hindering others (Callaway et al., 2003), or favoring the nutrient demands of particular functional groups of plants such as nitrogen (N) fixing species or species with enhanced phosphorus (P) acquisition through their association with mycorrhizal fungi (LeCroy et al., 2013; Quilliam et al., 2013; van de Voorde et al., 2014). Oram et al. (2014) reported increased competitive ability of legumes following biochar amendment as a result of increased potassium availability in a short-term pot experiment designed to emulate a semi-natural grassland. Alternatively, biochar may sorb root exudates that otherwise function as metal chelates released by plants to solubilize P (DeLuca et al., 2015b). Modification of soil P availability has been identified as a possible mechanism by which knapweed (Centaurea maculosa Lam.) outcompetes native plant species in North American rangelands (Thorpe et al., 2006; Zabinski et al., 2002). Biochar application to rangelands may alter plant-plant interactions via either short or long term modification of soil nutrient dynamics (Gao et al., 2019; Gao and DeLuca, 2016).

In much of the US Northwest, there are limited appropriate options for the handling of residual woody biomass from lumber mills or forest management activities (i.e. timber harvest). Generating biochar from pyrolysis of mill waste or forest residuals and applying it to nearby grassland or rangeland systems may therefore represent an opportunity to benefit wood waste utilization while facilitating soil C and nutrient management on site (McElligott et al., 2011). To date, there have been few studies conducted in western rangeland ecosystems to evaluate the influence of wood biochar on soil biota, nutrient cycling and nutrient retention. Given that biochar cannot be incorporated to depth in rangeland ecosystems, but rather are dependent upon physical and biological pedoturbation for incorporation, it is our expectation that the difference between agricultural and rangeland soil response to biochar will be related to the differences in incorporation depth and timing.

Herein, we conducted a biochar field study on a semi-natural rangeland ecosystem to evaluate how biochar, with or without organic fertilizer, affected soil N and P pools and fluxes over a one-year period. Arid and semi-arid rangeland ecosystems in the western USA are characterized by low yet variable precipitation with high evaporative demand that limits nutrient mobility in soils (Blank et al., 2007). Therefore, the objectives of our study were to evaluate how biochar addition to rangeland surface mineral soil would affect an array of indices directly associated with nutrient mobility, including: 1) Transformations involving reactive N production (i.e. N2 fixation), N conversion (i.e. nitrification), N consumption (i.e. nitrous oxide reduction to dinitrogen), and downward translocation of N in the soil profile; 2) The bioavailability of soil P and its downward translocation over one growing season. We targeted shifts in microbial N functional gene abundance to assess the relationship between microbial functional groups and N processing rates by measuring the abundance of genes that encode enzymes directing the rate-limiting steps in N cycling. We predicted that: 1) Adding biochar to a relatively productive rangeland soil would result in a limited effect on any of the microbial N processes or the inorganic N accumulation below surface soil as per previous findings (DeLuca et al., 2006); 2) Soil P bioavailability would be increased following biochar applications due to its direct "P fertilization" effect (Gao et al., 2019) and its positive effect on soil pH that has been reported elsewhere (Gao and DeLuca, 2018; Schaller et al., 2015), and the net accumulation of ortho-P below the surface mineral soil would be decreased in biochar-treated soils compared to the control (DeLuca et al., 2015b; Gao and DeLuca, 2019). The findings achieved in this study will help us better understand whether biochar can be used as a surface soil amendment in rangeland ecosystems to improve soil physical and biochemical conditions and processes.

2. Materials and methods

2.1. Site description and study design

A field study was initiated in the summer of 2018 (early August) at three independent sites (SSP: 47.05, -113.24; EPS: 47.07, -113.25; WPS: 47.07, -113.25) located at Bandy Experimental Ranch, Ovando, MT, USA. The region has a temperate continental climate, with an average annual precipitation of 400–460 mm. The wettest months are May and June (42–45 mm monthly average precipitation, also see Table S1), and December, January, and February are commonly the months of greatest snowfall. Growing season of the region is cool and short, the monthly average precipitation for July–September 2018 and 2019 was 25–30 mm. Mean temperature is 17 °C in July and -8 °C in January. The soils on the prairie portion of the ranch are predominantly Typic Haplocryolls derived from glacial till deposits (NRCS, USDA soil survey), with a gravelly sandy clay loam texture (~15% rock fraction, and 27% clay, 58% sand, and 15% silt in the mineral fraction).

Replicated treatment plots were laid out in a randomized block pattern (n = 3) at each of the three sites (N = 3). All plots at all three sites had similar aboveground species coverage dominated by Timothy-grass (Phleum pratense L.), but with the common presence of rough fescue (Festuca campestris Rydb.), Idaho fescue (Festuca idahoensis Elmer), smooth bromegrass (Bromus inermis Leyss.), yarrow (Achillea millefolium L.), wild geranium (Geranium maculatum L.), and several sedge species (Carex spp.). Since portions of the Experimental Ranch receive external organic fertilizer due to cattle farming with hay production, we included an organic fertilizer treatment in addition to biochar treatment in our field study. Specifically, a full factorial design consisting of biochar and organic fertilizer was established in each block where four treatments used in this study included: 1) Control with no additional amendment; 2) Organic fertilizer: a poultry litter based organic fertilizer (2:4:3 N–P–K) applied at 70 kg N ha⁻¹; 3) Wood biochar applied at 20 t ha⁻¹; 4) Charged biochar: biochar previously mixed with poultry litter before application (70 kg N ha^{-1} + 20 t ha^{-1}). Local irrigation water (containing no N or P) was used to create a slurry of dry organic fertilizer and biochar in Treatment 4 (soaking biochar in the poultry litter slurry), while the same volume of water was also applied to the control plots, and applied to the poultry litter in Treatment 2 and the biochar in Treatment 3 before treatment application to field plots (see Gao et al. (2016); Gao and DeLuca (2018) for more details). Each treatment plot was 2 m by 2 m in size with 1.5 m buffer in between. Treatments were applied to the surface soil and incorporated to approximate 5-10 cm depth with a rake and tines of a pitchfork. We also gently raked control plots to ensure consistency and protected aboveground vegetation from being destroyed by treatment application across all plots. The treatments were applied in early August 2018 with each treatment being randomly assigned to plots within each replication block, resulting in a total of 36 treatment plots across all three sites (i.e. four treatments in each block, three replicated blocks at each site, and three replicated sites). Biochar was produced using charred wood waste from lumber mills of F.H. Stoltze Land & Lumber Company (Columbia Falls, MT, USA) as a by-product from the electrical co-generation plant (https:// www.fhstoltze.com/; http://egenindustries.com/; https://genesisbioch ar.com/). The feedstock of wood biochar was a mixture of Douglas fir (Pseudotsuga menziesii L.), western larch (Larix occidentalis L.), grand fir (Abies grandis L.), subalpine fir (Abies lasiocarpa L.), and lodgepole pine (Pinus contorta L.). Biochar was press processed to 1-2 cm diameter before application. Charcoal generation temperatures were observed to be in the range of 450–550 °C (personal communication). Characteristics of both poultry litter and biochar are summarized in Tables S2 and S3. Three sites used in this study share similar background properties that are listed in Table 1.

Table 1

Soil physical and biochemical properties from the study site at Bandy Experimental Ranch, Ovando, MT, USA.

Parameter	Value
pH (1:1 v/v soil-to-DI water)	5.72 ± 0.40
Total C	$59.0 \pm 0.9 \ \mathrm{g \ kg^{-1}}$
Total N	$4.58 \pm 0.15 \ { m g \ kg^{-1}}$
NH_4^+ –N	$3.75 \pm 0.70 \text{ mg kg}^{-1}$
NO ₃ ⁻ -N	$0.12 \pm 0.03 \text{ mg kg}^{-1}$
Microbial biomass N	$182\pm11~\mathrm{mg~kg^{-1}}$
Potentially mineralizable N	7.70 \pm 1.67 g kg $^{-1}$ 14d $^{-1}$
Nitrification potential	137 ± 15 g NO $_3^-$ –N kg $^{-1}$ h $^{-1}$
CaCl ₂ –P	$4.95 \pm 1.41 \text{ mg kg}^{-1}$
Citrate-P	57.6 \pm 17.8 mg kg ⁻¹
Enzyme-P	$30.0 \pm 5.6 \text{ mg kg}^{-1}$
HCl–P	$289\pm23~\mathrm{mg~kg^{-1}}$

2.2. Soil sampling and analyses

Four surface soil subsamples (0-15 cm) were collected and composited to create a single sample from each treatment plot early (May) and late (September) in the growing season of 2019. Fresh soil samples were thoroughly homogenized and passed through a 2-mm sieve before being analyzed for a series of physicochemical and biochemical variables. Soil pH was determined on field-moist soil (1:1 v/v soil-to-DI water). Extractable NO₃⁻ –N and NH₄⁺ –N were determined by shaking fresh soil samples in 1M KCl for 30 min, filtering through Whatman 42 filter papers, and the extractants analyzed by microplatecolorimetric techniques using the vanadium-chloride method and salicylate-nitroprusside method, respectively (Mulvaney et al., 1996). Potentially mineralizable N (PMN) was measured using a 14 d anaerobic incubation method (Bundy and Meisinger, 1994). Briefly, 5 g of field moist soil was immersed in 15 ml DI water in a 50 ml polycarbonate centrifuge tube, the headspace was then displaced with N2 gas to eliminate oxygen and the centrifuge tubes were capped and incubated at 25 °C for 14 days. Samples were extracted by adding 15 ml of 2 N KCl (to create 30 ml of 1 N KCl extractant), shaken for 30 min, filtered and analyzed for NH⁺₄ using the method described above. Total PMN was calculated by subtracting initial NH₄⁺ (day 0) from that determined at the end of the incubation (day 14). Microbial biomass N (MBN) was determined by fumigation extraction method with amino-N determination by reaction with ninhydrin (Brookes et al., 1985). Nitrification potential (soil microbial potential to nitrify NH₄⁺) was determined on fresh soils using the aerated slurry method described by Hart et al. (1994). Soil P status was determined using the biologically based P (BBP) method which is designed to assess a suite of four plant P acquisition strategies to evaluate P bioavailability in dynamic soil systems (DeLuca et al., 2015a). Briefly, 0.01 M CaCl₂, 0.1 M citric acid, 0.2 EU ml^{-1} phosphatase enzyme, and 1 M HCl were used as extractants to emulate free soluble P, citrate extractable inorganic P that is weakly clay-sorbed or bounded in inorganic precipitates, labile organic P readily attacked by phosphatase enzymes, and moderately stable active inorganic P present in P-precipitates (DeLuca et al., 2015a). Oven dried (70 $^{\circ}$ C) soil samples were ground, sieved and analyzed for total C and N using a CHN analyzer (PE 2400 CHN Analyzer, Thermo Fisher Scientific, Waltham, MA, USA). Each composite soil sample was considered as an analysis unit (n = 36).

2.3. Soil DNA extraction and quantitative PCR

To better infer whether biochar exerted potentials to affect specific soil N processes (e.g. nitrification, nitrous oxide reduction, N₂ fixation by free-living N fixers in soils), the relative abundance of bacterial *amoA*, *nosZ*, and *nifH* genes were determined in soils collected both early and late in the 2019 growing season. Here we suggest that the relative abundance of soil *nifH* gene can provide insights on ecosystem N₂ fixation given that few leguminous species exist on our field plots; and both *amoA* and *nosZ* were chosen partially because they encode enzymes directing the rate-limiting processes in N cycling (Kuypers et al., 2018). Meanwhile, here in our study we anticipated to only examine the final step of complete denitrification and did not particularly consider the intermediate N processes producing NO or N₂O, given that we gave the assumption that these gaseous N forms were likely to be transformed into other reactive N forms and being tightly recycled within the soil biota before leaving the semi-arid N-limited ecosystem (Hooper and Johnson, 1999; Wedin, 1996). Numerous studies have also shown that biochar addition to soil could effectively reduce the production of NO or N₂O (Cayuela et al., 2014), we therefore only examined functional genes that were of specific interests to us here.

Total microbial genomic DNA was extracted from 0.25 g fresh soil samples using the QIAGEN DNeasy PowerSoil Kit following the manufacturer's instructions. The quality of the extracted DNA was checked using electrophoresis in agarose gels (1% w/v in TAE buffer) and the quantity was determined using a NanoDrop ND-1000 UV–vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Extracted soil DNA was then stored at -20 °C prior to further manipulation.

Quantitative PCR (qPCR) was used to assess the relative abundance of bacterial 16s rDNA, fungal ITS, and specific N functional genes (bacterial amoA encoding bacterial ammonia monooxygenase, nosZ encoding nitrous oxide reductase, and nifH encoding nitrogenase reductase) in soil samples collected at both early and late seasons of 2019. Primer sequences and qPCR thermal cycling conditions are listed in Table S4. All qPCR reactions were conducted on a Stratagene Mx3000P qPCR Machine (Thermo Fisher Scientific, Waltham, MA, USA), and all samples and controls (both positive and negative) were assayed in triplicate. Each qPCR reaction mixture (20 µl) contained 10 µl 2x iTaq Universal SyBR Green Supermix (Bio-Rad, Hercules, CA, USA), 0.5 µl of each primer, 3 µl of DNA, and 6 µl of Nuclease free water. At the end of each qPCR, melting curve analysis was performed to ensure the target product was generated, and the product was run on an agarose gel to confirm the correct size of specific target gene (16s 1500 bp, ITS 600 bp, amoA 491 bp, nosZ 454 bp, and nifH 458 bp). All the qPCR amplification data were auto-analyzed through the MxPro qPCR Software (Agilent Technologies) where a certain threshold cycle (Ct) was used as the detection limit for a specific target gene. The relative abundance of the target gene was calculated using the $\Delta\Delta$ Ct method where 16s rRNA gene was used for normalization (Livak and Schmittgen, 2001). A comparative Ct method (Pfaffl, 2004) was then used to calculate the fold change of the relative abundance of each target gene in treatments compared to the control treatment (fold change = $2^{\wedge}(-\Delta\Delta Ct)$).

2.4. Net accumulation of nutrients below surface soils

To determine how biochar with or without poultry litter would affect the net accumulation of nutrients below the surface mineral soil layer, we buried ionic-resin capsules (UNIBEST Ag Manager, mixed anion and cation resin, UNIBEST International, WA, USA) at approximately 25–30 cm soil depth at the center of each plot in early August 2018. The resin capsules were retrieved by the end of May 2019 after remaining in the soil for ten months. Nutrients captured in resin capsules were extracted sequentially with three 10 ml aliquots of 0.5*M* HCl (Gao et al., 2016) and analyzed for NO_3^- and NH_4^+ by colorimetric methods as described above, and P, Al, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, S, and Zn were measured using an inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific 6300, Waltham, MA) as described elsewhere (Soltanpour, 1991).

2.5. Statistical analysis

Soil data collected at early (May) and late (September) growing season of 2019 were analyzed and presented separately. An analysis of variance (ANOVA) and Tukey's post-hoc test were carried out on individual soil parameters to examine the significance of treatment effects with "treatment" serving as the fixed factor. "Site" and "replication block" both served as random factors before the fixed factor and were removed whenever significant effect not observed at P > 0.05. To better infer the statistical significance of each treatment (poultry litter, biochar, and charged biochar) to control with estimated uncertainty considered, we used the log response ratio (natural logarithm of treatment value divided by control value) and 95% of confidence interval (Ho et al., 2019) to present the effect sizes of treatments across individual soil biochemical variables. A principal component analysis (PCA) was performed on late-season soil data to elucidate the dominant patterns in soil characteristics and investigate major components driving the differentiation in soil processes one year following treatment

incorporation. Several soil variables were grouped or released to address our study interest and reduce the large number of explanatory variables for the PCA model (e.g. the geometric mean of four fractions of BBP was used in PCA as variable "BBP", geometric mean of resin NO_3^- and NH_4^+ was used in PCA as "N loss"). Significance for the PCA model, each axis and each variable, was tested using Monte Carlo randomization tests; and the variable loadings were presented by converting eigenvector coefficients to structure correlations (Legendre and Legendre, 1988). A permutation of analysis of variance (PERMANOVA) was conducted on late season soil data (where Euclidean distance was chosen) to test for differences in overall soil responses among treatments and controls. The significance of the *Pseudo-F* value was tested via 999 random



Fig. 1. Effects of poultry litter, biochar, or charged biochar on soil biochemical variables at (a) early or (b) late growing season of 2019 at Bandy Experimental Ranch, Ovando, MT, USA. Data are presented as logarithmic response ratios \pm 95% confidence intervals (n = 9). The response ratio is defined as the value of specific soil variable in treatment plot divided by that in control plot. Overlapped error bars indicate that treatments are not statistically significant from each other. *Abbreviations: MBN – microbial biomass nitrogen, PMN – potentially mineralizable nitrogen.*

permutations. All data were tested for homogeneity of variance and normality of residuals before analyses, and were log-transformed when necessary. All statistical analyses were performed in R (R Core Team, 2016).

3. Results

3.1. Soil biochemical responses

Biochar additions to a semi-natural grassland soil over a one-year period significantly increased soil total C and the availability of soil biologically based P (particularly citrate-P, enzyme-P, and HCl–P) regardless of whether biochar was used alone or in combination with poultry litter (Fig. 1). By contrast, the responses of soil N (NH₄⁺, NO₃⁻, MBN, and PMN) varied slightly across treatments and between seasons (Fig. 1).

During the early growing season of 2019 (late May), we observed a reduction in soil NH_{+}^{+} with an increase in soil MBN when biochar was applied alone, whereas no significant change was detected in NH_{+}^{+} or MBN when biochar was used with poultry litter (Fig. 1a). Soil NO_{3}^{-} response to treatments was variable with no overall treatment effects (over control) detected (Fig. 1a). Soils amended with poultry litter had relatively higher PMN, higher total N, and lower pH, while biochar had no significant impact on these three variables during the early season of 2019 (Fig. 1a, Table 2).

There was no significant change in soil total N, NH^{\pm}, or MBN one year after treatment applications during the late growing season (early September) sampling period (Fig. 1b, Table 2). Soil pH, however, increased significantly by 1.0–1.7 units by biochar addition when either comparing biochar to control or comparing charged biochar to poultry litter (Table 2). Biochar used alone significantly raised the pool size of soil NO₃⁻ as well as soil nitrification potential (Fig. 1b, Table 2). The charged biochar treatment resulted in significantly higher anaerobic 14d PMN compared to control soils (Table 2). It is also important to note that all four fractions of soil BBP were significantly higher in biochartreated soils during the late growing season of 2019 (Fig. 1b).

3.2. Bacterial 16s, fungal ITS, and N functional genes

We found that biochar additions had limited effect on the relative abundance of bacterial 16s, but a significant positive effect on the relative abundance of fungal ITS at both seasons (Fig. 2). This indicates a shift towards a fungal dominated microbial community in soils amended with biochar throughout the field trial. The relative abundances of the soil bacterial *amoA* gene were significantly higher in all three treatments compared to those in control soils in both early and late season, whereas there were no treatment effects detected on the abundance of either nitrous oxide reductase *nosZ* or nitrogenase *nifH* gene one year following treatment incorporation.

3.3. Net accumulation of nutrients below surface soil

The poultry litter treatment resulted in greater accumulation below surface soil (25–30 cm deep) for soil NH_{+}^{+} and P compared to that observed in control soils, and adding biochar to soil receiving poultry litter slightly reduced those accumulations below surface soil (Table 2, Fig. S1). Biochar applied alone generally had no effect on the net accumulation of most of the nutrients examined, except that soil P and S accumulations below the surface mineral soil layer were significantly increased compared to controls (Table 2, Fig. S1).

3.4. Relationships among soil variables

Much of the variance in soil responses was explained by the first two axes of the PCA model (a total of 48% explained, Table 3), where all soil variables included in the model had their structure coefficients greater than 0.55 and were significantly altered by biochar additions (Fig. 3; PERMANOVA comparing no biochar and biochar, *Pseudo* F = 10.1, P < 0.001). Overall, soil pH, NO₃, fungal ITS and *amoA* abundance, nitrification potential, and biologically based P were identified as parameters

Table 2

Soil physical, biochemical properties, and nutrient accumulation below surface soil as determined by accumulated nutrients in resin capsules at 25–30 cm depth in response to poultry litter, biochar, and charged biochar one year following additions to soil at Bandy Experimental Ranch, Ovando, MT, USA. Data are presented as mean \pm standard error (n = 3). Numbers with the same letter are not significantly different at P = 0.05 and no letter following the numbers indicate no significant differences among treatments at P = 0.05. Abbreviation: PMN – Potentially mineralizable nitrogen.

Soil variable	pН		Total C		Total N	PMN NO ₃ ⁻ -		NO ₃ ⁻ -N	NH ₄ ⁺ –N			Microbial biomass N			
Unit			${\rm g}~{\rm kg}^{-1}$				mg kg ⁻	$1 14d^{-1}$	$mg \ kg^{-1}$					mg kg ⁻¹	
Season	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	7	Late	Early	Late
Control	$\begin{array}{c} \text{5.72b} \pm \\ \text{0.50} \end{array}$	$\begin{array}{c} \text{6.60b} \pm \\ \text{0.18} \end{array}$	59.0c ± 0.9	60.7b ± 0.8	4.58b ± 0.15	$\begin{array}{c} \textbf{4.26} \pm \\ \textbf{0.20} \end{array}$	7.70b ± 1.67	8.70b ± 1.60	$\begin{array}{c} 0.12 \ \mathrm{ab} \\ \pm \ 0.08 \end{array}$	$\begin{array}{c} 0.30b \pm \\ 0.09 \end{array}$	3.75 0.70	b±	4.67 ± 1.24	$\begin{array}{c} 182b \pm \\ 11 \end{array}$	$\begin{array}{c} 100 \ \pm \\ 12 \end{array}$
Poultry litter	$\begin{array}{c} \textbf{5.14a} \pm \\ \textbf{0.14} \end{array}$	6.11c ± 0.10	$65.3b$ ± 1.2	$\begin{array}{c} 62.3b\\ \pm \ 2.3\end{array}$	$5.73a \pm 0.51$	$\begin{array}{c} 4.53 \pm \\ 0.55 \end{array}$	13.1a ± 1.59	8.50b ± 2.44	$\begin{array}{c} 0.07b \pm \\ 0.02 \end{array}$	$\begin{array}{c} 0.34b \pm \\ 0.04 \end{array}$	3.61 0.38	b±	$\begin{array}{c} \textbf{4.40} \pm \\ \textbf{0.04} \end{array}$	177b ± 23	$\begin{array}{c} 98.3 \\ \pm \ 8.7 \end{array}$
Biochar	6.17b ± 0.23	$7.57a \pm 0.17$	70.8a ± 4.4	$71.9a \pm 3.5$	4.85b ± 0.23	$\begin{array}{c} 4.35 \pm \\ 0.33 \end{array}$	6.21b ± 0.36	8.25b ± 1.56	$\begin{array}{c} \textbf{0.16a} \pm \\ \textbf{0.07} \end{array}$	$\begin{array}{c} \textbf{0.48a} \pm \\ \textbf{0.03} \end{array}$	2.45 0.31	a ±	$\begin{array}{c} \textbf{4.20} \pm \\ \textbf{0.85} \end{array}$	207a ± 13	111 ± 15
Charged biochar	6.39b ± 0.28	7.84a ± 0.19	69.5a ± 1.8	70.2a ± 1.4	5.70a ± 0.21	4.70 ± 0.13	13.7a ± 0.77	10.3a ± 3.40	0.15a ± 0.05	0.44a ± 0.10	3.31 0.61	b ±	$\begin{array}{c} \textbf{4.19} \pm \\ \textbf{1.14} \end{array}$	192ab ± 25	$\frac{102 \pm}{17}$
Soil variable	CaCl ₂ –P ble		Citrate-P Enzy		Enzyme-P HCl–P			Nitrification Resin potential NO ₃ ⁻ -N		Resin NH4 –N	Resin P				
	mg kg ⁻¹				$\begin{array}{c} g \ NO_3^ N \ kg^{-1} \\ h^{-1} \end{array} \qquad \mu g \ capsule^{-1} \end{array}$										
Season	Early	Late	Early	Late	Early	La	te	Early	Late	Late					
Control	$4.95b\pm1.41$	$\begin{array}{c} \text{5.30c} \pm \\ \text{3.84} \end{array}$	57.6c ± 17.8	173c : 49.1	± 30.00	l± 44 19	.7b ± .3	289b ± 22.9	444c ± 129	$138b \pm 25.4$		4.72 2.11	± 1	l5.9b ± l.72	41.4c ± 14.2
Poultry litter	$\begin{array}{c} \textbf{8.81a} \pm \\ \textbf{3.99} \end{array}$	8.94b ± 2.52	78.7b ± 16.4	530b 120	± 50.40 20.7	± 10 26	0a ± .4	320ab ± 18.2	$\begin{array}{c} 839b \pm \\ 67.8 \end{array}$	158 ab \pm 13.	.7	4.50 0.98	± 3	33.6a ± 9.80	98.0b ± 26.0
Biochar	$\begin{array}{c} \text{5.82b} \pm \\ \text{1.06} \end{array}$	$\begin{array}{c} \textbf{16.5a} \pm \\ \textbf{6.88} \end{array}$	$118a~\pm$ 6.99	850a : 24.2	± 75.41 11.7	0 ± 10 22	0a ± .0	375a ± 6.5	$\begin{array}{l} 1276a \pm \\ 69.1 \end{array}$	$186a \pm 18.3$		4.13 1.89	± 1	l 1.1b ± 2.33	$\begin{array}{c} 127a \pm \\ 9.03 \end{array}$
Charged biochar	$6.55a \pm 2.23$	$\begin{array}{c} 18.4a \pm \\ 6.86 \end{array}$	$139a \pm 6.80$	945a : 68.6	± 100a 6.30	± 92 34	.2a ± .1	378a ± 9.5	1386a \pm 97.6	$179a \pm 35.0$		3.25 1.05	± 1	l6.9b ± 5.93	86.3a ± 15.3



Fig. 2. Fold change in relative abundance of bacterial 16s, fungal ITS, bacterial *amoA*, *nosZ*, and *nifH* gene in poultry litter, biochar, or charged biochar over control at (a) early or (b) late growing season of 2019 at Bandy Experimental Ranch, Ovando, MT, USA. Data are presented as mean \pm 95% confidence intervals (n = 9). Overlapped error bars indicate that treatments are not statistically significant from each other.

Table 3

Structure correlation coefficients derived from principal component analysis of selected soil parameters measured in a biochar field study at Bandy Experimental Ranch, Ovando, MT, USA. Variables with coefficients <0.55 are not shown.

Variable	Axis 1 (35% explained, P < 0.001)	Axis 2 (13% explained, P < 0.05)	Axis 3 (12% explained, P < 0.1)
рН	0.65		-0.58
NO_3^-	0.64		
NH_4^+	-0.56		
N loss (resin N)		-0.55	
P loss (resin P)	0.59		
BBP	0.79		
ITS		0.64	
amoA	0.62		
Nitrification potential	0.65		
nosZ	0.65		

most sensitive to treatment incorporation among all variables examined in this study (Fig. 3). Soil pH had a relatively high structure coefficient (0.65) on axis 1 (and -0.58 on axis 3) and was highly positively correlated with nearly all variables pointing in the biochar and charged biochar direction. Among soil BBP, three out of four fractions showed high positive correlations with soil pH across treatments (Fig. 4). Soil NH⁺₄, as well as N net accumulation below surface biochar and mineral soil layer, were identified to be most sensitive to poultry litter addition, and were negatively correlated with the rest of the soil variables in the ordination space (Fig. 3, Table 3).



Fig. 3. Principal component analysis (PCA) ordination of selected soil biochemical and microbial parameters 1-yr following poultry litter, biochar, and charged biochar incorporation in a field study at Bandy Experimental Ranch, Ovando, MT, USA. Vectors (soil variables) sharing similar functions in shaping overall soil multifunctionality were grouped to reduce ordination complexity: N loss is the geometric mean of resin NO_3^- –N and resin NH_4^+ –N; BBP is the geometric mean of individual biologically based P (BBP) fractions. Vectors having structure coefficients <0.65 were excluded from the ordination plot.



Fig. 4. Correlations (Pearson's r, *P*-value) between soil pH and soil (a) CaCl₂–P, (b) Citrate-P, (c) Enzyme-P, and (d) HCl–P across treatments one year following field application at Bandy Experimental Ranch, Ovando, MT, USA.

4. Discussion

4.1. Response of soil nitrogen to biochar

The findings reported above imply no overall negative response in soil N availability following biochar application to a semi-natural rangeland soil of the US Inland Northwest. Despite a neutral to negative response of soil NH₄⁺ to biochar or charged biochar, soil NO₃⁻ concentrations responded positively to biochar and there was no significant increase in NH_4^+ or NO_3^- accumulation at 25–30 cm below the soil surface one year after biochar additions when compared to controls (Fig. 1, Table 2). The slight reduction in the soil NH_4^+ pool and an increase in soil microbial biomass N with biochar application at early season were most likely due to an elevated microbial C and N demand driven by biochar additions, given that wood biochar used here had a limited amount of labile C or N that would possibly trigger the microbial incorporation of C and N from resident organic matter (Kuzyakov, 2010). Contrary to our hypothesis, we observed a significant increase in nitrification potential, amoA abundance, and soil NO3 pool in late season in response to biochar, all of which provided strong evidence demonstrating that biochar used alone was able to help accelerate the conversion of NH₄⁺ to NO₃⁻ which might potentially benefit grass N uptake via diffusion and mass-flow (Davidson et al., 1990) and consequently, the N nutrition in this rangeland system. Previous studies using N isotopes have also demonstrated that Phleum pretense L., the dominant grass species in our study site, tended to have a higher NO_3^- absorption rate than NH_4^+ or glycine in natural grassland systems (Näsholm et al., 2000). Therefore, the reduction in soil NH⁺₄ with biochar additions and the finding that the soil NO_3^- pool did not increase with greater *amoA* abundance in the early season (Table 2) could also simply be a result of greater NO_3^- consumption by soil biota and active inorganic N uptake by grasses (Masclaux-Daubresse et al., 2010).

Previous studies conducted on forest or grassland soils have shown muted responses of the soil NO_3^- pool or net nitrification rate to biochar

additions either in situ or following NH₄⁺ addition in laboratory as substrate for nitrifiers. The authors argued that these soils showed little or no response to biochar additions, because the nitrifying communities were already highly active (Gao and DeLuca, 2019; MacKenzie and DeLuca, 2006). It is important to note that those studies were conducted on pH-neutral soils while the soil used in this study was somewhat acidic (Table 1) and soil pH was consistently raised by biochar additions (Fig. 3, Table 2) which may have stimulated the nitrifying community. Here, we argue that the shifts in soil pH with biochar applications move the soil towards a more optimal range for bacterial nitrifiers (Li et al., 2018; Xu et al., 2013) which are most likely responsible for the majority of N-related responses observed in our study (Fig. 3, Table 3). Wood biochar might have sorbed or reduced the activity of natural nitrification inhibitors (e.g. terpenes) thereby indirectly favoring nitrifying communities, similar to the findings reported for post-fire soil nitrifying communities interacting with fire-derived charcoal (Ball et al., 2010; DeLuca and Sala, 2006; MacKenzie et al., 2006). Greater soil porosity and moisture retention by biochar additions might simultaneously promote the substrate diffusion rate and thus the activity of nitrifying bacteria (Stark and Firestone, 1995). Alternatively, wood biochar itself might have directly acted as an "electron shuttle" (Saquing et al., 2016; Sun et al., 2017) that affected microsite redox potential and subsequently impacted the process of autotrophic nitrification.

Soils with higher nitrification rates also seemed to have higher N_2 production from N_2O potential (as inferred from the positive response of the relative abundance of *nosZ* encoding N_2O reductase) (Fig. 3). In our study, biochar or charged biochar generally had no significant effect on the potential of either the N loss through N_2O to N_2 or external N gain via biological N_2 fixation associated with free-living diazotrophs (Fig. 2), a finding consistent with our hypothesis and many previous studies (Xiao et al., 2019). We expected that there would be little NO or N_2O generation in response to biochar application despite of some occasionally wet conditions and given that accelerated nitrification would require aerobic conditions (Norton and Stark, 2011). It is somewhat surprising that the

accelerated nitrification in surface soils did not result in a greater accumulation of NO₃⁻ collected in resin capsules at 30 cm under the biochar treatments (Fig. 1, Table 2). It is possible that increased $NO_3^$ production in biochar-treated surface soils was directly taken up by plants and microbes or transformed to other forms via dissimilatory pathways with decreasing redox potential. Gaseous forms of N could be held in the pores of particulate biochar along the vertical movement, consequently resulting no significant differences in resin NO_3^- between control and biochar-treated soils. Overall, biochar applied alone to rangeland soils examined here could therefore result in a net neutral to a positive effect on N availability at the ecosystem scale over one growing season. Our study also showed that inorganic N accumulation below surface soils was most directly influenced by the addition of external N (organic fertilizer) rather than biochar (Fig. 3). However, charged biochar increased PMN (Table 2) and nitrification (Fig. 2) possibly by providing both sufficient substrate (NH_4^+) and optimal pH (and others) for nitrifying communities (Ouyang et al., 2016). Therefore, wood biochar charged with organic fertilizer could be a promising approach in conserving C while retaining organic N inputs and promoting inorganic N availability on acidic temperate rangeland soils.

4.2. Response of soil phosphorus to biochar

Our study demonstrated that wood biochar used alone strongly increased soil P bioavailability, but inconsistent with our hypothesis, also increased the net accumulation of available P on ionic resins buried below surface mineral soil (Fig. 1, Table 2). This combined with our observation of increased sulfur accumulation on resins at 30 cm (Fig. S1) suggests that the anion exchange capacity (AEC) of wood biochar used here might not increase phosphate (PO_4^{3-}) or sulfate (SO_4^{2-}) retention in surface soils (Taghizadeh-Toosi et al., 2011). It is also possible that the AEC of biochar might be significantly reduced over one year of biochar physiochemical "aging" in soil, which was demonstrated in previous studies conducted on cellulose biochar produced at \sim 500 °C, similar to what was used in our study (Lawrinenko et al., 2016; Lawrinenko and Laird, 2015). Alternatively, macropores created with particulate biochar applications might have allowed vertical transport of anions (Major et al., 2010), where PO_4^{3-} and SO_4^{2-} were found to accumulate in resins whereas NO_3^- underwent transformations as argued above. It is interesting to note that soil BBP fractions were consistently higher in the late season than those in the early season (Fig. 1, Table 2). This might be directly associated with a relatively lower P demand of plants and microbes towards the late growing season, which was partially in alignment with a significant drop in microbial biomass at the late season (Table 2, and a lower concentration of total extracted DNA, data not shown). We also observed a much smaller particle size for most biochar residing in soils collected in the late season, compared to fresh biochar added at the beginning of the field trial, these biochar might exhibit higher surface area and faster surface processes (Ameloot et al., 2013) creating additional positive effects on soil water holding capacity, P mobilization processes (e.g. solubilization from insoluble inorganic P, or mineralization from resident organic matter), and consequently P availability (Gao et al., 2017; Gao and DeLuca, 2018). Given that the study site was not found to be specifically limited by P (Black, 1968; Thorpe et al., 2006), it is not surprising to find that the biochar-induced increase in soil BBP was not retained within the system.

The charged biochar treatment promoted the retention of P while similarly increasing the BBP in topsoils (Figs. 1 and 3, Table 2). This finding might be associated with some spectroscopic and microscopic evidence reported elsewhere showing that the total capacity for a charged biochar to retain anions was significantly higher than that of organic fertilizer alone, non-charged biochar, or those two numbers simply combined (Joseph et al., 2018). The wood biochar used in this study contains almost no N, but some P (Tables S2 and S3), thus when used alone may significantly lower bulk soil or *charosphere* soil N:P. Resident soil microbial communities would consequently exert a low N: P recycling pattern where N would more likely to be immobilized (Fig. 1) while P would be lost from the system (Table 2), according to the consumer-driven nutrient recycling theory (Zechmeister-Boltenstern et al., 2015). Similarly, soil N supply is more likely to match soil P supply when biochar is charged or applied with an organic fertilizer causing P to be retained in surface soils prior to being assimilated.

It is important to note that the BBP content of biochar only accounted for $\sim 0.1-1.1\%$ (on a mass/area basis) of the total soil BBP when treated with biochar (Table S3). Therefore, biochar may have indirectly stimulated soil P availability and possibly shuttled some of the "temporary unavailable P" to "bioavailable P" pools (Gao and DeLuca, 2018). An increased enzyme-P pool under biochar could simply be associated with an accelerated microbial turnover with the microbial necromass containing labile organic P (Turner et al., 2005). Similarly, citrate- and HCl-P pools (inorganic P weakly to moderately sorbed to clay particles and precipitates) both positively responded to biochar additions regardless of additional organic fertilizer (Fig. 1), where biochar functional groups could have been involved in ligand-exchange reactions releasing some P from other "unavailable" P pools (Chintala et al., 2014). Alternatively, more P was desorbed from "unavailable" pools along a shifting soil pH (Schneider and Haderlein, 2016) that was altered by biochar (Fig. 4). Interestingly, the positive biochar effect on enzyme-P disappeared when charged with an organic fertilizer at the late growing season (Fig. 1b, Table 2). This may be due to a lower capacity for biochar to adsorb and retain resident organic P compounds, when biochar either resides in soil over a longer period of time or has an existing coating (e.g. organic fertilizer) on its surface reducing the ability to sorb others.

A significant positive soil P response and a relatively neutral soil N response one year following biochar addition (Table 2) might have contributed to the shift towards a fungal dominated microbial community (Fig. 2). Previous studies conducted on grassland soils have found a relatively lower mean molar N:P ratio for fungi than bacteria (Mouginot et al., 2014) and reported a negative relationship between soil N:P ratio and fungal:bacterial ratio (de Vries et al., 2006). Here, the fungal community would be expected to have a higher relative P requirement than the bacterial community (Sterner and Elser, 2002), however, this assumption contradicted findings in other studies (Güsewell and Gessner, 2009; Zhang and Elser, 2017). Biochar might have directly promoted the fungal mycelial networks favoring grass rhizosphere processes (Hammer et al., 2014), particularly in the late season when biochar became smaller in particle size that might support better soil moisture retention. The response of fungal community could also simply be a side-effect of plant root responses to biochar, where the optimal moisture and pH ranges that were adjusted by biochar provided signals towards physiological changes in roots, and consequently their fungal partners (Kammann and Graber, 2015). Nevertheless, it remains unknown whether the response of microbial community or soil N or P to biochar observed here in the first growing season following amendment remains stable over the years or how plant community composition or ecosystem function respond to biochar over long term.

4.3. Implications for management

The addition of biochar alone or charged with a poultry litter slurry to an acidic, semi-natural temperate rangeland soil stimulated soil nitrification without further increasing inorganic N accumulation below surface soils. And although biochar significantly increased surface soil BBP, the total soluble P collected on resins at 30 cm depth was also promoted when biochar was applied without an N source. This effect on net P accumulation below surface soil could be reduced by incorporating the fertility source to biochar prior to application, where charged biochar amendment strongly increased the bioavailability of P with no significant effect on accumulation of P at depth likely partially due to a shift in soil pH due to the ash associated with the biochar. Clearly, applications of agricultural lime (CaCO₃) to soils is a more efficient means of achieving an increase in pH in acidic agricultural or rangeland soils; however, the soil pH increase achieved with wood biochar addition to rangeland soils is only one of numerous potentially positive effects of biochar on soil properties and processes (soil moisture relations, improve physical condition, nutrient retention, microbial activity) not accomplished by liming agents. Further, lime applications must be made regularly where biochar can be viewed as a single or occasional soil amendment.

Charged biochar also helped retain soil PMN and total N and promoted nitrification without noted accumulation of inorganic N at depth. These results indicate that wood biochar charged with an organic fertilizer (or biochar used on soil patches receiving an internal nutrient source, e.g. livestock waste) represent an alternative to composting and could work efficiently at retaining soil nutrients and promoting soil C storage in a semi-natural rangeland system.

The lack of agricultural byproducts in this region, combined with the common need for woody fuel reduction makes biochar generation from woody residues an effective alternative to compost as a high C soil amendment. Storage of biomass C as biochar that would otherwise be commonly considered waste and likely pile burned in the region could provide an array of abiotic and biotic benefits that may prove differentially valuable depending on the site constraints and that year's conditions. Unlike inorganic fertilizer, biochar does not provide a single, static benefit for given set of time, rather it imparts a change in the physiochemical character of surface soils that may increase N availability when moisture is abundant (Gao et al., 2016), or improve moisture retention during a drought year (Ali et al., 2017), or increase nutrient retention when moisture is in excess (Jeffery et al., 2017). In a fertile agricultural ecosystem, wood biochar may not induce an N response, but instead promote soil moisture and the retention of other nutrients (e.g. P, Fe, Ca) in the short term (Gao et al., 2017; Gao and DeLuca, 2018). In a less fertile rangeland ecosystem, wood biochar may participate in various soil internal nutrient cycling processes that over time may increase the mobility of soil N and P and potentially benefiting biological nutrient assimilation. This variable benefit makes biochar a long-term investment rather than an annual treatment to achieve a specific nutrient objective. The lack of tillage in rangeland ecosystems create challenges for the implementation of this practice, but when combined with intensive grazing regimes, resulting localized manuring and biopedoturbation would potentially improve the efficacy of such treatments.

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.

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Appendix A. Supplementary data

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