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Rangeland application of biochar and rotational grazing interact to influence soil and plant nutrient dynamics

Si Gao[a,b,*], Thomas H. DeLuca[c]

[a] Department of Life & Environmental Sciences, School of Natural Sciences, University of California, Merced, CA 95343, United States
[b] Department of Forest Engineering, Resources & Management, College of Forestry, Oregon State University, Corvallis, OR 97331, United States
[c] Department of Forest Ecosystems & Society, College of Forestry, Oregon State University, Corvallis, OR 97331, United States

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ABSTRACT

Few studies have been conducted to evaluate the use of wood biochar in temperate rangeland ecosystems and none have investigated the interactive influence of wood biochar and cattle trampling activity (associated with short-term rotational grazing) on soils and plants. We conducted a field study on a semi-natural pasture in western Montana, USA to evaluate how wood biochar, with or without short-term intensive trampling affected biochar incorporation depth, soil physicochemical properties, nutrient dynamics, and grass nutrient concentrations with a particular focus on nitrogen (N) and phosphorus (P). We hypothesized that cattle trampling alone would induce soil compaction and generally reduce soil nutrient cycling rates and/or availability, but the presence of biochar prior to trampling could improve soil aeration and grass nutrition. After three months, we found that trampling alone significantly increased surface soil bulk density and reduced the values of nearly all soil N metrics examined in this study; however, the application of biochar prior to trampling improved infiltration rate and increased net nitrification. Trampling also facilitated biochar incorporation into soils. Biochar additions significantly increased soil available organic P (i.e., enzyme extractable P) while reducing soil inorganic P (i.e., $CaCl_2$-P, citrate-P) with or without trampling. Soil P responses appeared to be associated with biochar ortho-P sorption capacity and was more pronounced in soils that had undergone cattle trampling. Changes in soil P were reflected in grass P concentration after three months. Overall, our field study demonstrated that the use of biochar on rangeland soils with short-term rotational grazing could result in a neutral to positive effect on soil and plant nutrients.

1. Introduction

Wood biochar production and application to soils has been promoted as an effective way to make use of forest residuals generated in fuel reduction efforts, while increasing soil carbon (C) sequestration, improving soil moisture and nutrient retention, and alleviating nutrient leaching potential (DeLuca and Gao, 2019; Gao et al., 2017). However, to date, few studies have investigated the influence of wood biochar on soil processes in managed rangeland ecosystems that receive little disturbance compared to tillage and harvest activities in agricultural crop production (Gao and DeLuca, 2020; Phillips et al., 2020; van de Voorde et al., 2014). In the US Northwest, there are limited appropriate options for the handling and use of residual woody biomass from forest management activities (i.e., timber harvest or fuel reduction treatments). Generating biochar from woody residuals at forest management sites or mill operations and applying it to nearby agricultural, forest, prairie or rangeland systems represents a realistic opportunity for wood waste utilization while improving soil C storage and nutrient management.

The lack of tillage in rangeland ecosystems reduces the capacity for biochar to be incorporated to depth in mineral soil, instead requiring physical and biological pedoturbation for soil mixing (Gao and DeLuca, 2020; Phillips et al., 2020). It is our expectation that the activities of cattle on rangeland sites will modify soil responses to biochar addition (e.g., associated with incorporation depth and intensity) in comparison to sites without the presence of cattle (Stavi, 2012). Previous studies have reported that cattle trampling associated with continuous or rotational grazing on pastures can result in soil compaction (Byrnes et al., 2018; Taboada and Lavado, 1993) and that changes in the hydraulic and mechanical properties of soil (Somerville et al., 2020) can

* Corresponding author at: Department of Life & Environmental Sciences, School of Natural Sciences, University of California, Merced, CA 95343, United States.
E-mail address: sgao14@ucmerced.edu (S. Gao).

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lead to changes in soil microbial activity (Kohler et al., 2005), microbial community composition (Hiltbrunner et al., 2012), and nutrient transformation rates (Bhandral et al., 2007). Similarly, numerous studies have demonstrated the capacity of biochar to alter nutrient cycling rates (e.g., nitrification, phosphorus mineralization) via a range of abiotic (e.g., soil bulk density, soil porosity, biochar sorption capacity) and biotic (e.g., microbial activity) mechanisms (DeLuca et al., 2015b; Gao and DeLuca, 2019; Makoto and Koike, 2020). To our knowledge, no studies have evaluated the interactive effect of biochar and cattle trampling associated with short-term rotational grazing on biochar incorporation depth, soil water relations, nutrient dynamics, as well as grass nutrition in rangeland ecosystems.

Rotational grazing is a common and viable fenced grazing system for livestock production and rangeland management. In comparison to continuous grazing, rotational grazing can reduce grazing pressure on localized habituated areas, stimulate grass growth, and induce a spatially more heterogeneous manure distribution that will subsequently provide a positive feedback on grass and livestock productivity (di Virgilio et al., 2019; Jacobo et al., 2006). Previous studies have found that short-term rotational grazing generally had a neutral to positive effect on soil C storage and fertility in spite of increasing soil bulk density in comparison to no grazing (Byrnes et al., 2018; Warren et al., 1986). Given the physical properties of biochar and its widely-documented nutrient retention capacity, it is possible that the soil incorporation of biochar prior to rotational grazing and associated cattle trampling will relieve some of the soil compaction pressure (Karim et al., 2020; Liu et al., 2017) and reduce downward migration of nutrients and subsequently improve nutrient retention and grass growth in the ecosystem (Gao and DeLuca, 2021).

Herein, we conducted a three-month field study on a semi-natural rangeland ecosystem to evaluate how biochar, with or without a 3-day intensive cattle trampling event, influenced the depth of biochar incorporation into soil, soil physicochemical properties, nutrient dynamics, and grass nutrient concentrations with a particular focus on nitrogen (N) and phosphorus (P). We hypothesized that: (1) Cattle trampling alone would lead to soil compaction and reduce soil aeration which subsequently reduce soil nitrification rate (Bhandral et al., 2007; Mulholland and Fullen, 1991), but the presence of biochar prior to trampling could improve soil aeration that would favor nitrifiers (Gao and DeLuca, 2020; Pietola et al., 2005); (2) Cattle trampling associated with intensive grazing would help incorporate biochar into the mineral soil where a greater portion of biochar surface area would interact with soil matrices (Joseph et al., 2010) reinforcing the effect of biochar on soil P bioavailability (Gao et al., 2019; Gao and DeLuca, 2018); (3) An increase in net nitrification with biochar with or without cattle trampling might also exert a positive influence on soil P availability by reducing the N limitation during the process of microbial phosphatase enzyme production (Moorhead et al., 2016; Wang et al., 2007); (4) Changes in soil N or P in response to biochar and trampling would be reflected in grass nutrition (Gao et al., 2016). The findings achieved with this study will help us better understand whether wood biochar can be used in conjunction with short-term cattle trampling and grazing in US western rangeland ecosystems to improve soil physical and biochemical conditions.

2. Materials and methods

2.1. Study site description

A three-month field study was initiated in May 2020 at Bandy Experimental Ranch, Ovando, Montana, USA (47°03′57″ N, 113°15′32″ W). The region has a temperate continental climate, with an average annual precipitation of 400 – 460 mm. The wettest months of the year are May and June (42 – 45 mm monthly precipitation). Growing season of the region is cool and short, mean temperature is 17 °C in both July and August. The soils on the prairie portion of the ranch are predominantly loamy-skeletal, mixed, superactive Typic Haplocryolls (Mollisols, parent material: alluvium) (NRCS Soil Survey Staff, 1999). Detailed soil properties are given in Supplemental Table S1. All field plots have 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), and smooth bromegrass (*Bromus inermis* Leyss.).

2.2. Field trial design

Replicated treatment plots were laid out in a randomized block, split-plot pattern involving biochar amendments and cattle trampling as part of a rotational grazing scheme (Fig. 1). Six replicated grazing paddocks (12 m × 120 m) were set up where cattle trampling was randomly assigned to three paddocks and no cattle was assigned to three paddocks and labeled ‘no trampling’. The entire experimental area was cordoned off with electrical fence to deter other grazing ungulates from entering the experiment. Each paddock was also fenced to ensure cattle trampling was a controlled activity and an appropriate treatment. Within each paddock, we installed nine treatment plots where each of three biochar treatments (control with 0 t ha^{-1} biochar, 20 t ha^{-1} biochar, and 40 t ha^{-1} biochar) were replicated three times and all treatments were randomly assigned to the nine plots (Fig. 1). Overall, there were 54 plots, 27 treatment plots were established within ‘no trampling’ paddocks and 27 treatment plots were established with ‘cattle trampling’ practice. Each treatment plot was round-shaped with 1 m^2 in size (0.56 m as the diameter) and had approximately 10 m buffer in between.

2.3. Biochar and cattle trampling

Biochar treatments were applied to the surface soil in early May 2020 and incorporated to approximately 3 cm depth with a rake and tines of a pitchfork. We also gently raked control (0 t biochar ha^{-1}) plots to ensure roughly an equivalent amount of disturbance across all plots. 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. 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.). All biochar used in this study was stored in a dry, cool storage location for approximately one year following production at the pyrolysis unit before being applied to the field. Biochar was press processed to 1–2 cm diameter particles before application. Biochar production temperatures were reported to be in the range of 450 – 550 °C (personal communication), chemical characteristics of biochar were determined prior to field application and are summarized in Supplemental Table S1. A three-day intensive cattle trampling was initiated in late June where cattle were let in the grazing paddocks at a rate of 24 heifers per paddock for three continuous days. The trampling and grazing scheme here is considered a “high-intensity, low-frequency” management practice that is periodically adopted on semi-natural grasslands of the region to stimulate regeneration (www.attra.ncat.org). Here we consider the physical process of trampling and the associated manure input by cattle (approximately 4.2 kg N and 2.8 kg P per paddock, equivalent to 29 kg ha^{-1}N and 19 kg ha^{-1}P) to be the only noted influences on soils during the three-day period of on-site cattle activity of our study and consider any other ancillary influences on soils to be negligible.

2.4. Soil sampling and analyses

Infiltration rate measurements were carried out in the field for all treatment plots using the single ring infiltrometer method following Chowdary et al. (2006). Six surface soil subsamples (0 – 15 cm) were collected and composited to create a single sample from each treatment plot three months following biochar addition (mid July 2020, two weeks

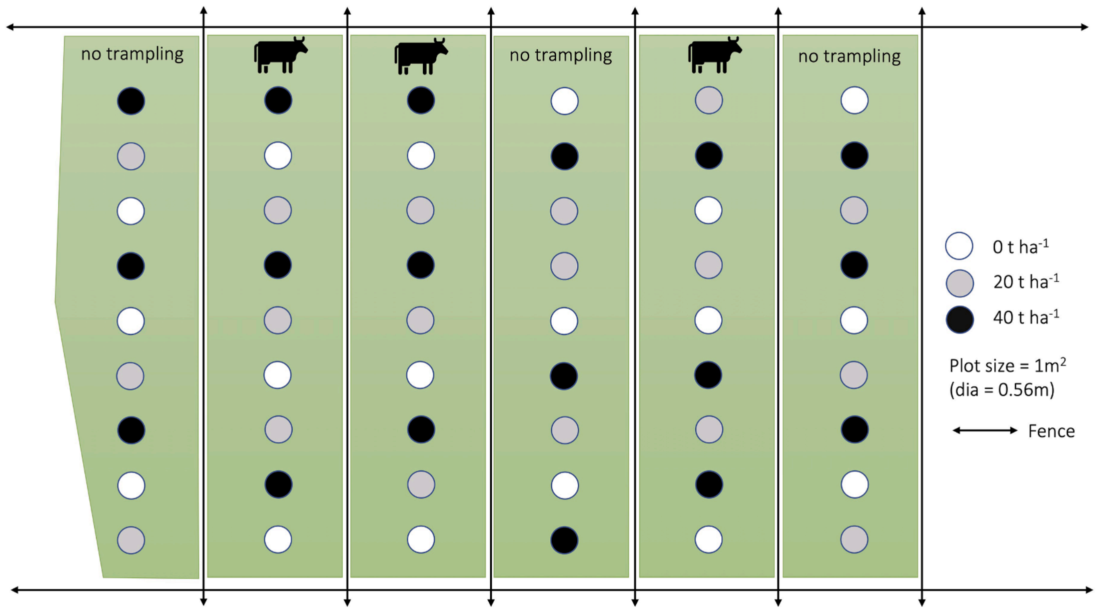

Fig. 1. Experimental layout for a three-month biochar field trial at Bandy Experimental Ranch, Ovando, Montana, USA (note not to scale). Control (0 t ha^{-1}) or biochar treatments (20 and 40 t ha^{-1}) were randomly assigned to 1 m^2 plots (n = 9) within each 12 m × 120 m paddock (N = 6); three paddocks were randomly chosen to go through an 3-d intensive cattle trampling treatment following biochar application.

following the 3-d intensive cattle trampling). To determine whether cattle trampling helped incorporate biochar into the soils, each of the six sampling cores (subsamples) were first visually assessed for the maximum depth at which biochar pieces appeared in the soil core and the depths were recorded with a measuring tape. Visible root tissue was removed from soil samples prior to any subsequent soil sieving or drying. Fresh soil samples were thoroughly homogenized and passed through a 2-mm sieve before being analyzed for a series of physico-chemical and biochemical variables. Bulk density was determined using a bulk density core (10 cm height × 7 cm diameter) that was pressed into the soil in the field. Water holding capacity was determined by gravimetry (Loveday, 1974). Soil pH was determined on field-moist soil (1:1 v/v soil-to-DI water). Oven dried (70 °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).

2.5. Soil N and P concentrations

Extractable NO_3^- -N and NH_4^+ -N were determined by shaking fresh soil samples (5 g oven dried equivalent) in 30 ml 2 *M* KCl for 30 min, filtering through Whatman 42 filter papers, and the extracts then analyzed by microplate-colorimetric techniques using the vanadium-chloride method for NO_3^- and salicylate-nitroprusside method for NH_4^+ (Mulvaney et al., 1996). Microbial biomass N (MBN) was determined by fumigation extraction method with amino-N determination by reaction with ninhydrin (Brookes et al., 1985). Net ammonification and net nitrification were determined on fresh soils using the 30-d aerobic incubation method described by Hart et al. (1994).

Soil P status was determined using the biologically based P method which was designed to assess a suite of four plant P acquisition strategies to evaluate P bioavailability in dynamic soil systems (DeLuca et al., 2015a; Pingree et al., 2017). Briefly, 0.01 *M* $CaCl_2$, 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). Each composite soil sample was considered as an analysis unit (total n = 54).

2.6. Biochar nutrient sorption capacity

Biochar pieces were manually picked from soil samples after the three months field trial to determine the capacity for inorganic N or ortho-P sorption. Ten to 15 biochar particles were picked and separated from the composite soil sample that was collected from individual treatment plot. Nearly all biochar pieces collected were approximately 1 cm in diameter and we therefore did not intentionally separate biochar samples by particle size. The inorganic N and ortho-P sorption capacity for biochar samples were determined following the protocols described in Takaya et al. (2016) and Yao et al. (2012). Briefly, biochar particles were gently washed with DI water prior to the batch sorption experiment. Batch solutions were prepared as 1000 mg L^{-1} NH_4^+ -N solution (NH_4Cl), 100 mg L^{-1} NO_3^- -N solution (KNO_3), and 125 mg L^{-1} PO_4^{-3} -P solution (KH_2PO_4). Approximate 0.1 g biochar samples were added to batch solutions and the mixtures were shaken at 160 rpm for 24 h at room temperature (Pingree et al., 2016). We had previously conducted pilot batch sorption experiments on these biochar samples and found that 24 h and 48 h of shaking generated similar results, therefore we considered a 24 h shaking period as adequate to reach equilibrium and provide comparable results in our current study. Aliquots of supernatant were then taken following the 24 h shaking and equilibrium and filtered through 0.45 μm syringe filters and subsequently analyzed for inorganic N and ortho-P analyses as described above. The concentration of sorbed ions for biochar (sorption capacity) were determined as:

$$I_s = (C_0 - C_{24}) \times \frac{V}{M}$$

where I_s is the sorbed ions (mg g^{-1}) C_0 and C_{24} are the initial (0-h) and equilibrium (24-h) liquid-phase NH_4^+, NO_3^-, or PO_4^{-3} concentrations, respectively; V is the volume of the solution; and M is the mass of biochar. The same set of protocols were applied to both field-collected biochar samples and to biochar not applied in the field.

2.7. Grass tissue nutrient concentrations

A single species, *P. pratense*, was selected for sampling because of its common presence in all plots. Grass foliar tissue samples were clipped and collected from each treatment plot by the end of the field trial.

Samples were taken back to lab, washed with DI water, dried in oven and triturated in a domestic food processor resulting in a homogeneous texture and mass. The dried tissue was then analyzed for total N on a CHN analyzer as described above. Total P and other macro and micro nutrients (e.g., K, Na, Ca, Mg, Fe, Mn, Zn) were determined using an inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Scientific 6300, Waltham, MA) following a dry-ashing and nitric acid digestion procedure (Zarcinas et al., 1987).

2.8. Statistical analysis

Analysis of variance (ANOVA) and Tukey's post-hoc tests were carried out on individual soil metrics or grass nutrients to examine the significance of biochar, trampling, and their interactive effects with biochar and trampling both served as fixed factors. Replicated paddocks served as a random factor before the fixed factor and were removed whenever effect was not significant ($P > 0.05$). Nonmetric multidimensional scaling (NMDS) analysis was performed on all soil and biochar metrics to elucidate the dominant patterns in soil characteristics and investigate major components driving the differentiation in soil processes three months following treatment incorporation with or without the 3-d intensive trampling event in order to recognize and interpret patterns that represent the underlying ecological gradients. Variables were continuous where Euclidean distance was selected for the NMDS model and all data used in the NMDS model were log transformed to reduce skewness and the influence of outliers. We selected the first two NMDS axes (dimensions) to display for practicality, and adding more dimensions was not found to significantly reduce the stress value. The NMDS was performed using the function metaMDS() from the "vegan" package (number of dimensions was set to 2). Significance for the NMDS model, each axis, and each variable, were tested using Monte Carlo randomization tests (Legendre and Legendre, 1988). The variable loadings (i.e., variable weights) on each derived axis was generated using the function envfit() along with the NMDS scores (library "vegan" in R). Pearson correlation tests were then conducted on selected variables that were of interest to us to examine the relationships between biochar sorption properties and selected soil metrics. A permutation of analysis of variance (PERMANOVA) (with Euclidean distance) was conducted on soil N data or P data to test for differences in grouped N or P responses among treatments and controls. The significance of the *Pseudo-F* value was tested via 999 random permutations. When necessary, we used mixed linear regression (MLR) model to determine dominant drivers controlling selective soil metrics in this field study. All data were tested for homogeneity of variance and normality of residuals before the MLR analyses and were log-transformed when necessary. All statistical analyses were performed in R (R Core Team, 2020). Procedures of statistical analyses and data visualizations were adopted from R packages described in Sarkar (2008), Wickham (2016), Oksanen et al. (2019), and Sievert (2020).

3. Results

3.1. Soil physicochemical properties

In this three-month biochar field trial we found that the inclusion of an intensive cattle trampling and grazing treatment significantly increased the depth to which biochar was incorporated into the mineral soil (biochar was found to a depth of 3.9 – 4.5 cm without trampling and to depths of 7.4 – 9.0 cm with trampling, P (trampling) < 0.001, Table 1). Soils that had undergone cattle trampling on control plots without biochar had significantly higher soil bulk density ($P < 0.001$) and had lower infiltration rates ($P < 0.05$) compared to soils without trampling (Table 1). The presence of biochar significantly reduced the impact of trampling on soil bulk density ($P < 0.01$) and improved soil infiltration rates ($P < 0.001$) and water holding capacity ($P < 0.001$) (Table 1). Soil total C was significantly increased by biochar additions

Table 1
Soil metrics (0–15 cm) in response to biochar application at 20 t ha^{-1} or 40 t ha^{-1} with or without an intensive-cattle-trampling event associated with rotational grazing at Bandy Experimental Ranch, Ovando, Montana, USA. Data are presented as mean ± standard error (n = 9). Values followed by the same letters are not significantly different at $P = 0.05$. No letters following the values indicate no significant difference at $P = 0.05$.

Cattle	Biochar	Bulk density (g cm^{-3})	Biochar depth (cm)	Infiltration rate (cm h^{-1})	Water holding capacity (t ha^{-1})	pH	Total C (t ha^{-1})	Total N (t ha^{-1})	$CaCl_2$-P (kg ha^{-1})	Citrate-P (kg ha^{-1})	Enzyme-P (kg ha^{-1})	HCl-P (kg ha^{-1})
No trampling	Control	0.84b ± 0.03	N/A	10.2b ± 2.46	812b ± 20.5	6.9 ± 0.1	61.3c ± 4.0	4.8 ± 0.5	82.2a ± 4.57	623.5a ± 45.3	200.1ab ± 6.72	1127 ± 108
No trampling	20 t ha^{-1}	0.60c ± 0.03	3.9c ± 0.4	17.4a ± 1.78	888a ± 27.6	7.0 ± 0.1	65.2bc ± 5.6	5.0 ± 0.7	47.1bc ± 5.83	414.5b ± 22.4	195.5b ± 18.2	1000 ± 80.4
No trampling	40 t ha^{-1}	0.66c ± 0.06	4.5c ± 0.4	12.9ab ± 1.83	920a ± 58.4	7.2 ± 0.2	75.8ab ± 7.4	5.8 ± 0.5	48.6bc ± 6.17	425.7b ± 20.8	214.0a ± 4.20	1278 ± 134
Trampling	Control	1.11a ± 0.11	N/A	5.66c ± 0.78	773b ± 21.5	6.7 ± 0.3	58.9c ± 3.4	5.4 ± 0.4	69.0ab ± 10.2	538.8a ± 18.9	192.4b ± 7.48	1069 ± 92.5
Trampling	20 t ha^{-1}	0.63c ± 0.04	7.4b ± 0.3	13.2ab ± 2.26	862a ± 54.9	6.9 ± 0.2	81.7b ± 5.0	5.5 ± 0.2	43.4c ± 5.23	391.2b ± 32.9	222.8ab ± 14.9	1036 ± 142.4
Trampling	40 t ha^{-1}	0.69c ± 0.05	9.0a ± 0.7	11.4b ± 1.12	833ab ± 45.0	7.1 ± 0.2	102a ± 9.5	5.9 ± 0.2	44.2c ± 1.71	432.4b ± 26.6	267.2a ± 11.5	1198 ± 39.5

($P < 0.001$), but only modestly influenced by trampling ($P = 0.1$). Soil total N was not modified by either biochar applications or cattle trampling (Table 1).

3.2. Soil N and P concentrations

Soil N was generally more responsive to the 3-d intensive cattle trampling event than biochar treatment in this three-month field trial (Figs. 2 and 3, PERMANOVA for soil N metrics: P (biochar) > 0.1 and P (trampling) < 0.01). Trampling had a significant and consistently negative effect on soil net ammonification rate, NH_4^+ -N, microbial biomass N, and net nitrification rate, whereas trampling had a significant positive effect on extractable soil NO_3^- -N, regardless of biochar addition (Fig. 2). In trampled plots, the presence of biochar significantly increased soil net nitrification rates (Fig. 2d); however, the increased nitrification rate did not translate to an increase in soil NO_3^- -N (Fig. 2e) partially due to the fact that soil NO_3^- -N content in cattle trampling plots was already rather high (e.g., 10 kg ha^{-1}).

Extractable soil P was generally more sensitive to biochar treatments than the intensive cattle trampling (Table 1 and Fig. 3, PERMANOVA for soil P metrics: P (biochar) < 0.05 and P (trampling) > 0.1). Biochar additions at both 20 and 40 t ha^{-1} significantly reduced soil $CaCl_2$-P and citrate-P whereas biochar additions increased enzyme-P regardless of the occurrence of cattle trampling (Table 1 and Fig. 4). The reduction in soil citrate-P was also significantly correlated with an increase in biochar ortho-P sorption capacity as well as the increase in soil enzyme-P (Figs. 3 and 4). Additionally, cattle trampling associated with rotational grazing had a positive interaction with biochar application at increasing soil enzyme-P (P (trampling × biochar) < 0.1, Table 1 and 3, Fig. 3). Variation in soil enzyme-P was found to be partially explained by biochar ortho-P sorption, changes in soil citrate-P, water holding capacity, as well as net ammonification rate (Table 3). It is important to note that the two biochar application rates (20 t ha^{-1} and 40 t ha^{-1}) used in this study had similar effects on most of the soil properties and processes examined in this study (Fig. 3, PERMANOVA for all soil metrics comparing 20 t ha^{-1} and 40 t ha^{-1}, $P = 0.7$).

3.3. Biochar nutrient sorption capacity

The NH_4^+ sorption capacity for biochar collected in the field was not significantly influenced by cattle trampling ($P > 0.1$) or biochar application rate ($P = 0.08$) in spite of being relatively higher than that for the fresh biochar (Table 2). Field collected biochar from both 20 t and 40 t biochar ha^{-1} plots had significantly higher NO_3^- sorption capacity than that of a fresh biochar over the three-month period (Table 2); however, the NO_3^- sorption capacity of biochar did not lead to any shift in soil NO_3^- availability (Fig. 2) nor was sensitive to cattle trampling ($P = 0.05$). By contrast, the ortho-P sorption capacity for field-collected biochar was not only significantly higher than that of fresh biochar (Table 2), but also was responsible for changes in soil P availability in this three-month field trial (Table 3 and Fig. 3).

3.4. Grass nutrient concentrations

Grass N concentration was not significantly influenced by any treatment in this three-month field trial (Fig. 5a). The presence of biochar alone in plots without cattle trampling significantly reduced grass P concentration whereas the involvement of intensive cattle trampling with biochar significantly increased grass P concentration (Fig. 5b). Regardless of trampling, biochar addition at 20 t ha^{-1} significantly increased the grass Fe concentration and the grass Mn concentration was significantly increased by biochar at 40 t ha^{-1} (Fig. 5c, d).

4. Discussion

The results from this study demonstrated that cattle trampling as part of a short-term intensive rotational grazing scheme significantly reduced soil infiltration rate and subsequently reduced the size and rate of N pools or fluxes (e.g., microbial biomass N, net ammonification rate); however, the presence of wood biochar helped alleviate some of these negative effects of trampling. Biochar particle size was likely reduced and biochar particles incorporated to a greater depth of soil in plots with trampling and biochar additions prior to cattle trampling reduced soil compaction, facilitating soil aeration and infiltration, and increased water holding capacity and net nitrification rate (Table 1, Figs. 2 and 3). These findings demonstrate the potential short-term benefits of using biochar on rangeland sites with high probabilities of intensive herbivore trampling and support our first hypothesis that trampling would increase soil compaction which would be partially alleviated by biochar applications.

Cattle trampling seemed to have a more noted effect on soil N cycling than the addition of biochar (Fig. 3). For instance, most of soil N metrics

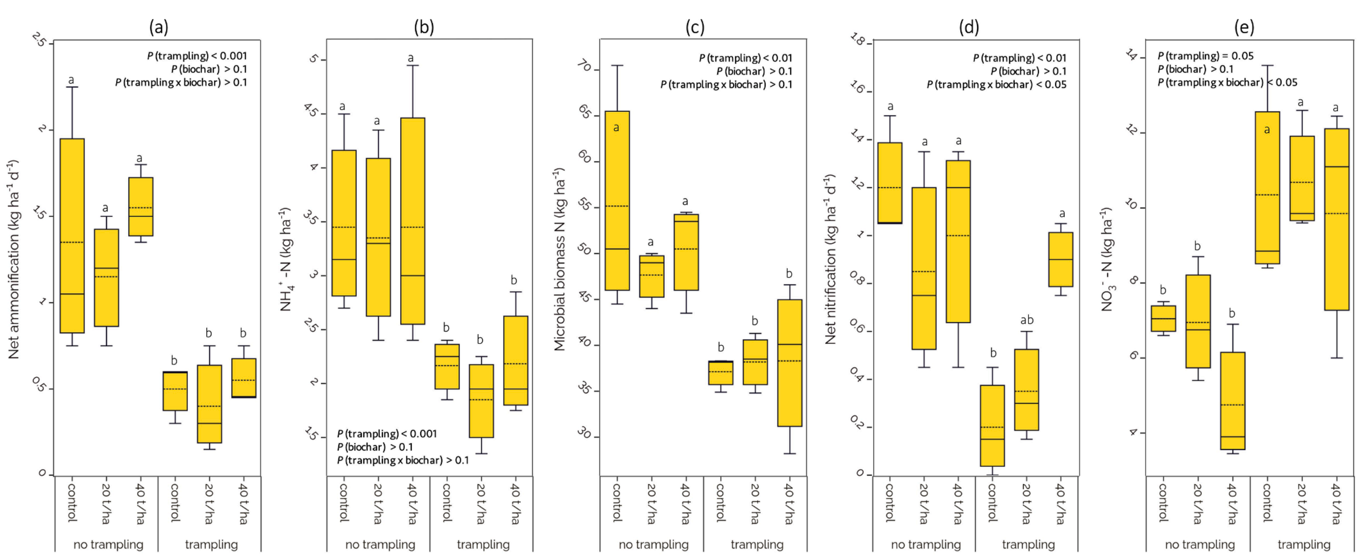

Fig. 2. Soil (a) net ammonification rate, (b) extractable NH_4^+ -N, (c) microbial biomass N, (d) net nitrification rate, and (e) extractable NO_3^- -N as influenced by biochar applications at 20 t ha^{-1} or 40 t ha^{-1} with or without a 3-d intensive cattle trampling event associated with rotational grazing in a three-month field trial at Bandy Experimental Ranch, Ovando, Montana, USA. Data were compared using Tukey-HSD test following ANOVA. Solid line indicates median, dashed line indicates mean. Columns with the same letter are not significantly different at $P = 0.05$.

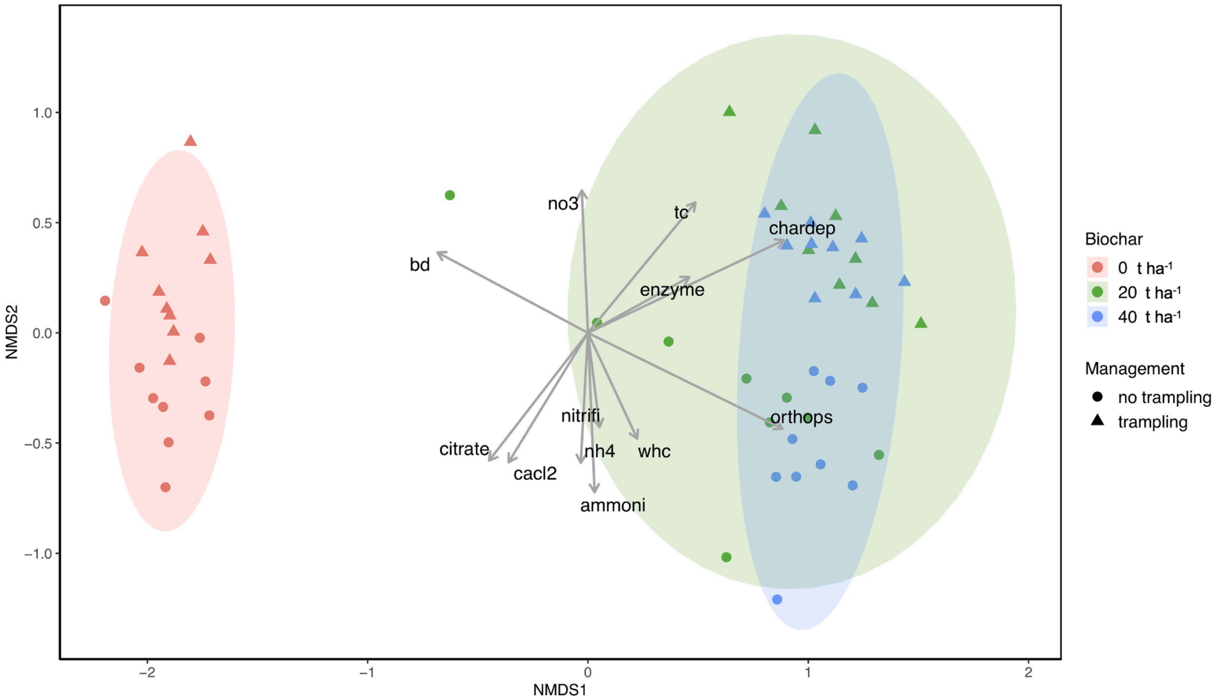

Fig. 3. Nonmetric multidimensional scaling (NMDS) ordination (stress = 0.08, non-metric fit R-squared = 0.99, linear fit R-squared = 0.98) demonstrating soil metrics in response to biochar addition (grouped by color) and cattle trampling event associated with rotational grazing (grouped by shape) in a three-month field trial at Bandy Experimental Ranch, Ovando, Montana, USA. *Abbreviations: bd = bulk density, tc = total C, chardep = depth of biochar identified in soils, orthops = biochar ortho-P sorption capacity, whc = water holding capacity, ammoni = net ammonification rate, nitrifi = net nitrification rate.*

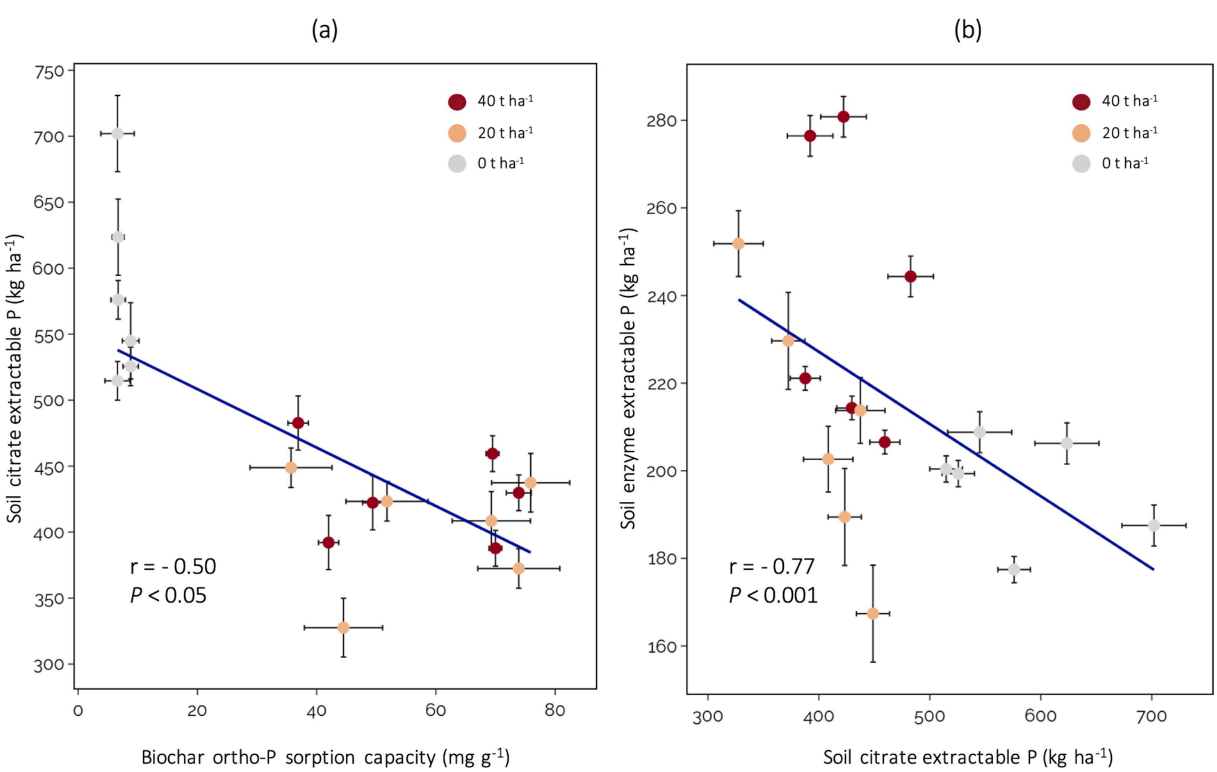

Fig. 4. Correlations (Pearson's r, *P*-value) between soil citrate extractable P (kg ha^{-1}) and (a) biochar *ortho*-P sorption capacity (mg g^{-1}) and (b) enzyme extractable P (kg ha^{-1}) at control (0 t ha^{-1}), 20 t ha^{-1}, and 40 t ha^{-1} biochar field plots in a three-month biochar and grazing field trial at Bandy Experimental Ranch, Ovando, Montana, USA. Note that the ortho-P sorption capacity for biochar at control plots in panel (a) indicates the ortho-P sorption capacity for biochar samples prior to field application. Data are presented as mean with standard error, where each data point represents a paddock-level value for individual treatment that accounts for within-paddock variation (n = 3 for each data point).

Table 2
Inorganic N and ortho-P sorption capacity for biochar samples collected from treatment plots both before and at the end of the field trial. Plots were treated with biochar at 0 t ha^{-1}, 20 t ha^{-1}, or 40 t ha^{-1} with or without an intensive-cattle-trampling event associated with rotational grazing at Bandy Experimental Ranch, Ovando, Montana, USA. Values followed by the same letters are not significantly different at $P = 0.05$.

Biochar source	NH_4^+ sorption (mg g^{-1})	NO_3^- sorption (mg g^{-1})	Ortho-P sorption (mg g^{-1})
Fresh, prior to field application	79.00b ± 8.39	2.50c ± 0.29	7.37c ± 0.72
No trampling 20 t ha^{-1}	96.80ab ± 23.6	3.77ab ± 0.56	53.80ab ± 11.1
No trampling 40 t ha^{-1}	127.7a ± 4.33	6.83a ± 0.17	71.13a ± 1.39
Trampling 20 t ha^{-1}	126.9a ± 19.0	6.53ab ± 1.79	62.23ab ± 9.56
Trampling 40 t ha^{-1}	73.87b ± 3.10	4.37b ± 0.32	42.77b ± 3.63

Table 3
Model statistics for enzyme extractable P regressed against biochar, trampling, and soil and biochar metrics in a mixed linear model (model fit R-squared = 0.98, adjusted R-squared = 0.95, *P*-value < 0.001). All data were log transformed in the model to ensure data normality and homogeneity of variance. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns indicates $P > 0.1$.

Coefficients:	t-value	*P*-value	Level of significance
Intercept	11.2	1.01e−5	***
Biochar (20 t ha^{-1})	−4.45	0.0029	**
Biochar (40 t ha^{-1})	0.76	0.4696	ns
Trampling	5.60	0.0008	***
Biochar (20 t ha^{-1}) × Trampling	−7.06	0.0002	***
Biochar (40 t ha^{-1}) × Trampling	−10.8	1.27e−5	***
Water holding capacity	−3.71	0.0075	**
Net ammonification rate	−3.13	0.0166	*
NH_4^+-N	2.32	0.0537	ns
Citrate-P	−8.59	5.75e−5	***
Biochar ortho-P sorption	2.87	0.0240	*

in plots with cattle trampling remained at significantly lower values than trampled plots in spite of the presence of biochar and the potential introduction of 29 kg ha^{-1} of manure N with the trampling treatments (Fig. 2). Net nitrification rate was significantly lower in trampled plots compared to no-trampling plots and was increased in the presence of biochar only in trampled plots (Fig. 2d). It is possible that the trampling-induced soil compaction impacted the physical environment (e.g., aeration) for nitrification. This is particularly true for clay textured rangeland soils as found in this study, which can limit oxygen availability during wet seasons (Schrama et al., 2013). It is therefore reasonable to assume that the improved soil aeration conditions associated with the biochar additions in turn influenced soil net nitrification rates (Ulyett et al., 2014). Contrary to the finding from our previous rangeland biochar study (Gao and DeLuca, 2020), we found no effect of biochar on soil pH. Soil pH was found to remain near 7.0 in plots before and after biochar additions and with or without cattle trampling (Table 1). It is possible that the duration of our experiment and the high buffering capacity of the soil limited our detection on bulk soil pH changes. Increased net nitrification in trampled plots with biochar was more likely a result of increased aeration than a result of changes in soil pH associated with biochar. It is important to note that the soils at this study site already exhibited high concentrations of inorganic N (and possibly NO_3^--N leaching potential), possibly due to the legacy effect of the frequent use of this area for calving (associated with years of concentrated high protein hay feeding) prior to this field trial (Table S1, Fig. 2b, e). The introduction of cattle on the plots further elevated the NO_3^--N levels possibly via their manure/urine input (Fig. 2e). Thus, it is not surprising that the increased net nitrification associated with biochar and cattle trampling did not translate to any additional changes in soil NO_3^--N. Similarly, the inorganic N sorption capacity of biochar did not lead to any significant changes in soil inorganic N levels, suggesting a lack of sufficient organic coating or mineral associations on biochar surface that would otherwise facilitate N retention in this three-month field trial (Hagemann et al., 2017; Mia et al., 2017). Although we did not measure soil NO_3^--N leaching potential in our current study, we believe that the addition of biochar would not contribute to additional NO_3^--N leaching loss in cattle-trampled plots at our study site (Knowles

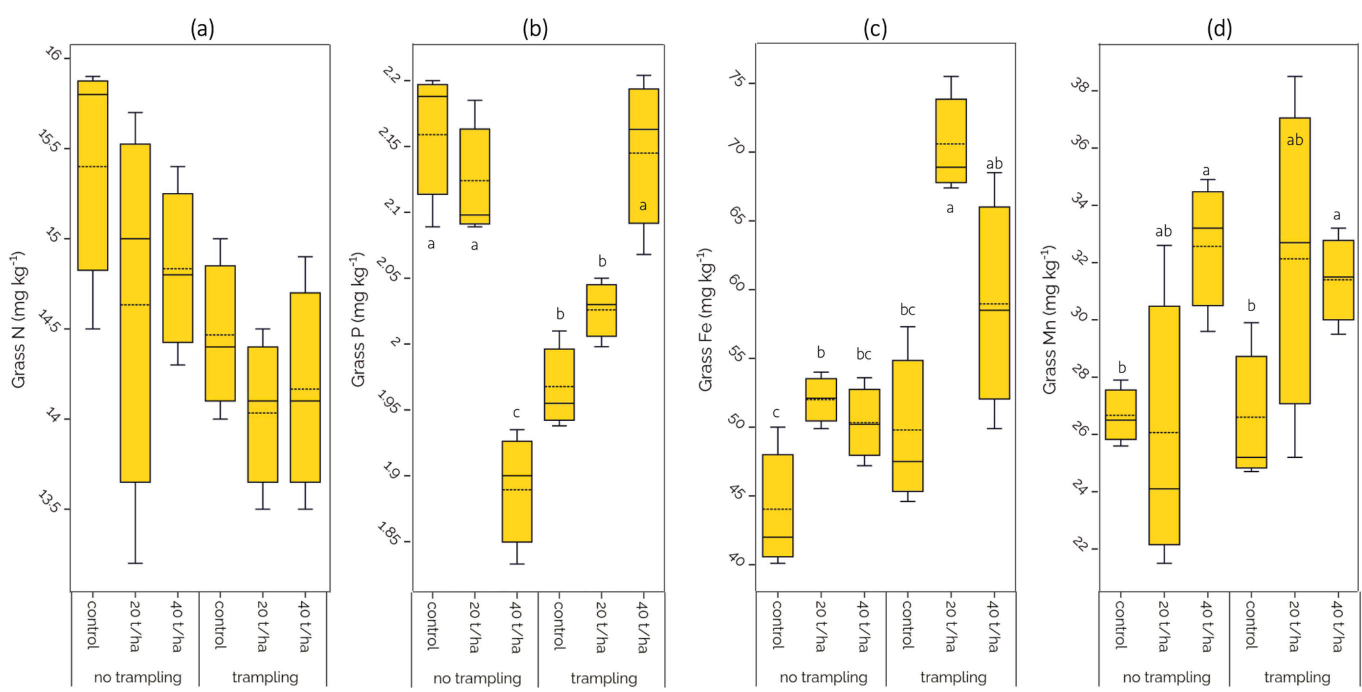

Fig. 5. Grass (a) N, (b) P, (c) Fe, and (d) Mn concentrations in response to biochar addition with or without a 3-d intensive cattle trampling event associated with rotational grazing in a three-month field trial at Bandy Experimental Ranch, Ovando, Montana, USA. Data were compared using Tukey-HSD test. Solid line indicates median, dashed line indicates mean. Columns with the same letter are not significantly different at $P = 0.05$.

et al., 2011). Soil nutrient pools and fluxes are often found to be a function of the spatial distribution of soil microsites (Leij et al., 2002; Sihi et al., 2020) which helps explain the dissimilarity of the field based observations from our studies to the results commonly reported in controlled laboratory studies.

Our study indicates that trampling helped incorporate biochar to a greater soil depth than in plots with no trampling in keeping with our second hypothesis. The increased depth of biochar with cattle trampling possibly led to greater biochar surface interactions with soil matrices reinforcing the ortho-P sorption and subsequent reduction of soil $CaCl_2$-P and citrate-P (Fig. 3, Table 1 and 2). However, it is important to note that the combined use of biochar and intensive cattle trampling did not impose any significant overall negative impact on soils in this study and the reduction in soil inorganic P in trampled plots receiving biochar did not seem to limit grass P nutrition (Fig. 5). However, the foliar P concentration could be indicative of P reallocation rather than P uptake. The lower soil inorganic P levels associated with the presence of biochar did appear to promote soil enzyme-P availability regardless of cattle trampling (Figs. 3 and 4, Table 3). This set of soil P responses to biochar and cattle trampling is possibly a function of an increased production of plant or microbial phosphatase enzyme (Ptase) associated with an increased demand for P under conditions of sufficient N and improved water retention conditions (Figs. 3 and 4, Table 3). These findings partially support our third hypothesis (i.e., an increase in net nitrification with biochar may exert a positive influence on soil P availability by reducing the N limitation during the process of microbial phosphatase enzyme production) and may provide a new mechanistic understanding (e.g., biochar ortho-P sorption) on how biochar influences rangeland soil P availability in addition to findings in our previous study (Gao and DeLuca, 2020).

The surface ortho-P sorption capacity of biochar could be related to the ash component of the biochar which in turn could be responsible for the observed reduction in soil inorganic P availability (Glaser and Lehr, 2019; Lawrinenko et al., 2016). Specifically, the P removal process could be a result of metal ion precipitation reactions between inorganic P in soils and alkaline metals (e.g., Ca, Mg) affiliated with the biochar (Takaya et al., 2016). This P removal mechanism by sorption was found to be particularly efficient for wood biochar on neutral to alkaline P-rich soils (Bornø et al., 2018) similar to that investigated in our study. It is well established that the activity and production of Ptase at the organismal scale can be controlled or induced by a low availability of inorganic P (i.e., supply–demand imbalance) as well as supplies of other limiting resources such as water and N (McGill and Cole, 1981; Treseder and Vitousek, 2001). Our study demonstrated a similar pattern where the increase in soil enzyme-P availability appeared to be controlled by biochar ortho-P sorption capacity, a decrease in citrate-P, and an increase in net ammonification and water holding capacity (Table 3). Cattle trampling following biochar applications could have driven the positive P responses by increasing the biochar-soil surface interactions (Makoto et al., 2011) for the soil ortho-P removal (Morales et al., 2013), Fig. 3 and Table 3). The increase in grass P concentration in biochar with cattle trampling plots also indicates that soil P responses might have resulted in higher plant P uptake (Fig. 5). Importantly, we found that the foliar N:P ratio in trampled plots was significantly lower with biochar treatments (especially at 40 t ha^{-1}) than in control plots (data not shown). Changes in foliar P concentration could also indicate reallocation of P in the rangeland grasses. It is possible that the addition of biochar induced an “N dilution effect” where P, rather than N, was preferentially reallocated to foliar tissues at higher biochar application rate, a result that is consistent with the findings of Gale and Thomas (2019).

The use of biochar with or without intensive grazing significantly increased grass Mn and Fe concentrations (Fig. 5). This finding is consistent with previous agricultural biochar studies and it is well established that these transition metals are in higher concentrations in biochar compared to native soils (Gao et al., 2017; Ippolito et al., 2015). Contrary to our final hypothesis, grass N concentrations did not reflect changes in soil N metrics in response to biochar or cattle trampling (Fig. 2 and Supplemental Table S1). However, we did find that short-term changes in dominant available soil P forms in response to biochar with and without cattle trampling were reflected in changes in grass P concentration over the three-month period (Fig. 5 and Table 1). The reduction in grass P concentration following biochar application paralleled the reduction in soil inorganic P in response to biochar addition to plots without cattle trampling. This is partially consistent with our previous study where we found that the use of biochar alone on similar rangeland soils increased soil ortho-P captured in ionic resins buried 30 cm below the soil surface (Gao and DeLuca, 2020). Alternatively, this reduction in foliar P concentration at the high biochar rate (40 t ha^{-1} without cattle) could reflect plant ecophysiological responses that were previously demonstrated to be rate-dependent (Gale and Thomas, 2019). Similarly, intensive grazing alone significantly reduced grass P concentration (Fig. 5). However, implementing biochar prior to cattle trampling improved grass P concentration as well as soil enzyme-P in comparison to cattle trampling alone or biochar alone (Fig. 5). The positive interactive effect of cattle and biochar on grass nutrition (i.e., biochar resulted in a decrease in grass foliar P concentration without cattle trampling, but resulted in an increase in grass foliar P concentration in trampled plots, particularly for the 40 t ha^{-1} biochar addition) could partially be attributed to a change in soil water relations or increased disintegration of biochar under intensive cattle trampling (Spokas et al., 2014). Despite the high infiltration rates in soils with biochar additions, it is possible that diffusive P leaching with biochar reduced grass P concentrations. Cattle trampling could have partially alleviated this negative impact by suppling P and by compacting surface soils temporarily reducing infiltration rates (Novak et al., 2016). The increased disintegration of biochar with trampling might increase biochar-soil surface interactions (as argued above), where higher biochar surface area associated with smaller particle size biochar could have sorbed more inorganic P (reducing $CaCl_2$-P, citrate-P) while stimulating enzyme-P (Fig. 4), subsequently driving changes in plant nutrient responses (Dodd and Sharpley, 2015). It is important to note that the biologically available soil P (Table 1) was one-time measurement that represented soil P status at a single timepoint whereas foliar P represents P uptake integrated over time. It is therefore not surprising that there was a mismatch between soil P responses and grass P responses in our field study as soil P responses could have been short-lived.

5. Conclusion

This study provides key observations on the interactive effects of intensive rotational grazing and biochar additions on soil nutrient dynamics. We found that cattle trampling associated with intensive rotational grazing significantly reduced N availability and turnover and generally had a greater impact on soil N than the biochar treatment alone. Biochar treatments had a more pronounced positive impact on soil P than intensive grazing by significantly improving soil enzyme-P in these organic matter rich surface soils. The 3-d intensive cattle trampling significantly improved incorporation of biochar into mineral soils compared to biochar applied without a trampling event. In comparison to trampling alone, the presence of biochar prior to trampling increased infiltration rates, reduced soil bulk density, and increased the soil net nitrification rate (perhaps due to greater aeration). Further, cattle trampling on soils amended with biochar increased soil enzyme-P and improved grass P nutrition in comparison to biochar additions without trampling.

These findings demonstrated potential short-term benefits of using wood biochar on temperate semi-natural rangeland soils exhibiting high probabilities of subsequent intensive animal trampling with short-term rotational grazing during the growing season. The incorporation of biochar into mineral soils with the help of cattle trampling may also bring additional benefits for soil C storage in rangeland ecosystems that commonly exhibit deeper rooting depth and less subsoil disturbance compared to row crop agroecosystems (Li et al., 2021; Lorenz and Lal, 2014). However, it is important to note that these findings in our study may not be expected over longer term (e.g., over three months), or in similar ecosystems, but with different trampling/grazing intensities or schemes (e.g., number and weight of animals per given area, continuous or rotational). High rate biochar applications (such as the 40 t ha^{-1} in our study) may also significantly reduce soil bulk density and create operational difficulties on soil incorporation of biochar. The cost of biochar production, transportation, and field application also needs to be taken into account when considering applying biochar to rangelands. Nevertheless, our study suggested that 40 t ha^{-1} biochar had a short-term negative effect on grass foliar P concentration when used alone; while the 20 t ha^{-1} and the 40 t ha^{-1} rates generally resulted in similar short-term responses of soil and plant nutrient dynamics when associated with cattle trampling and grazing. This finding implies that the higher biochar rate (40 t ha^{-1}) may have been excessive when considering the effect on grass P and the lower rate appears to achieve similar short-term benefits with regard to soil nutrient availability and plant nutrient uptake. Intensive rotational grazing on temperate semi-natural rangeland ecosystems of the US Inland Northwest has the potential to improve the efficacy of rangeland biochar applications by facilitating incorporation while the biochar improves soil physical properties associated with rotational grazing.

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115572.

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