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REGULAR ARTICLE

Soil amendments alter plant biomass and soil microbial activity in a semi-desert grassland

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Abstract
Aims We tested the effects of soil biotic disturbance and biochar or woodchip amendments on plant growth, soil microbial biomass and activity, and soil physiochemical parameters in response to disturbance in a semi-desert grassland.
Methods In a 78-day growth chamber experiment using six grass species native to the Southwest U.S., we compared the effects of autoclave heatshock, which mimics soil stockpiling in hot drylands, and amendments on plant and microbial biomass, potential extracellular enzyme activity, and soil moisture and nutrient availability.
Results Plant biomass was lowest in woodchip-amended soils, and highest in autoclaved and biochar-amended soils ($p < 0.05$). Root:shoot ratios were higher in the autoclaved and woodchip-amended soils ($p < 0.05$). Biochar addition improved soil water-holding capacity resulting in higher dissolved organic carbon ($p < 0.001$) and nitrogen ($p < 0.001$). Soil microbial activity and plant biomass were not correlated. Amendment-induced changes in activity could be partially explained by nutrient availability. Neither microbial biomass nor activity recovered to pre-disturbance values.
Conclusions In this study, biochar and woodchip amendment and autoclave-induced changes to moisture and nutrient availability influenced plant biomass allocation and soil microbial activity. Amendments increased carbon, nitrogen, and phosphorus mineralizing enzyme activities with no significant change in microbial biomass, indicating that soil recovery in drylands is a long-term process. Understanding plant-soil feedbacks in drylands is critically important to mitigating climate and anthropogenic-driven changes and retaining or reestablishing native plant communities.

Keywords Autoclave heat-shock · Biochar · Drylands · Extracellular enzyme activity · Plant-soil feedbacks · Woodchips

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Introduction

Arid and semiarid grasslands (drylands) represent one-third of the terrestrial land surface and store approximately 15% of terrestrial organic carbon (C) (Asner

et al. 2003; Reynolds et al. 2007). These globally predominant ecosystems face many anthropogenic disturbances, including urbanization and land use change, increased intensity of cattle grazing, and mineral extraction through mining (Bestelmeyer et al. 2015). These disturbances fundamentally alter species composition, rates of primary productivity, and nutrient cycling (Harris 1966; Jenerette et al. 2006). In Southwest U.S. drylands, projected warmer and drier conditions with more variable precipitation (Garfin et al. 2013; Pachauri et al. 2014) have the potential to further disrupt the function and stability of this region through direct effects on soil microbiota and altered plant-microbe interactions (Schlesinger et al. 1990; Anderson-Teixeira et al. 2011). Understanding the strengths and directions of feedbacks between these belowground and aboveground processes in dryland ecosystems is critically important to mitigating climate and anthropogenic-driven changes and retaining or reestablishing native plant communities.

The main factors influencing dryland ecosystem resiliency and potential for recovery include the frequency, timing, and duration of precipitation; soil physicochemical properties; and the composition of soil microbial communities (Belnap 1995; Huxman et al. 2004). Dryland plant species recruit only after sufficient seasonal rainfall (e.g. Fehmi et al. 2014; Moreno-de las Heras et al. 2016). Sufficient rainfall combined with soil water holding capacity and microbial community composition drive nutrient cycling that affects plant establishment. Heavily disturbed soils can limit both rainfall percolation and root penetration, which impedes plant establishment and growth (Bronick and Lal, 2005; Bindraban et al., 2012) and further promotes soil compaction and loss of aggregate structure. Soil disturbance lowers organic matter, reduces available ammonium (NH_4^+), nitrate (NO_3^-), phosphorus (P), and potassium (K), and results in depauperate microbial communities with lower potential function (Belnap 1995; Wong 2003). Soil stockpiling practices typical of mining projects in the Southwest U.S. cause large scale soil disturbances that can reduce soil microbial biomass (Visser et al. 1984). Autoclave heat-shock treatments can be used to mimic stockpiling practices by reducing, but not completely eliminating, microbial populations (Trevors 1996; Callaway et al. 2004).

Soil amendments are organic and inorganic materials added to a soil that become incorporated into the profile. Depending on their source and type, amendments have been shown to increase nutrient availability and organic matter quality, alter soil porosity and structure, and stimulate microbial biomass production, which may enhance plant establishment (Ehaliotis et al. 1998; Sohi et al. 2010; Ohsowski et al. 2012). Generally, amendments are added to a soil to improve its physical structure and nutrient status with an ultimate goal to increase plant growth and establishment (Davis and Whiting 2000). Organic materials such as biochar and woodchips are commonly used as amendments due to their availability and, in the case of woodchips, relatively low cost.

Pyrolysis of plant biomass results in biochar with extremely high surface area that quickly absorbs water and nutrients in soil, followed by slow release of nutrients over time (Atkinson et al. 2010; Artiola et al. 2012). Biochar also has the substantial advantage in that it may be eligible for economic subsidies as a method of C storage in addition to its potential for increasing biomass production (Lorenz and Lal 2014). The effects of biochar amendments on C and nitrogen (N) dynamics are varied and depend on the characteristics of the amendments and the system. For example, biochar additions can be associated with both an increase in N mineralization in forest systems (Berglund et al. 2004; DeLuca et al. 2006) and a decrease in N mineralization in agriculture and semiarid systems (Bruun et al. 2012; Dempster et al. 2012). The addition of biochar can alter the physical structure of soil, provide more surface area for microbial colonization, and affect microbial community activity and structure (Pietikäinen et al. 2000; Steinbeiss et al. 2009). Biochar addition also impacts nutrient dynamics in soils through their extremely high C:N and C:P ratios (Kookana et al. 2011) and their tendency to increase soil cation exchange capacity (CEC; Glaser et al. 2000; Cheng et al. 2008). Soils with higher CEC have an increased ability to hold and exchange nutrients such as NH_4^+, K, and calcium (Ca) (Brady and Weil 2008). Thus, by introducing organic C and N substrates, biochar addition can stimulate microbial growth in soils by increasing nutrient availability both quickly (after six days of incorporation into soils; Smith et al. 2010) as well as over longer time periods (up to 100 years) through initial nutrient retention and subsequent, gradual release (Zackrisson et al. 1996).

Managing woody shrub encroachment, a priority in Southwest ecosystems, can result in harvested woody biomass that could be used as woodchip soil amendments. Woodchips, which are mechanically chipped whole trees and shrubs, are often used to reduce soil moisture loss, regulate soil temperatures, increase soil

porosity, decrease compaction and erosion, and as a weed control agent (Kraus 1998; Downer and Hodel 2001; Chalker-Scott 2007; Sinkevičienė et al. 2009; Miller and Seastedt 2009). Woodchips have high C to N ratios, which may cause initial N-immobilization (Scharenbroch 2009). After a period of rapid decomposition, however, this nitrogen can be released from soil microorganisms and become available for plant growth (Miller and Seastedt 2009). Woodchips, from both hard and softwood sources, have been shown to increase permeability and retain soil water better than unchipped treatments (Kraus 1998; Davis and Whiting 2000; Chalker-Scott 2007; Gruda 2008). Woodchip effects on plant growth are varied in the literature with research showing both positive and negative trends (Davis 1994; Downer and Hodel 2001; Miller and Seastedt 2009). It is likely that the effects of woodchips on plant growth are system dependent and are impacted by the initial source of the woodchips and the degree of woodchip decomposition when applied (Davis and Whiting 2000). The direct and indirect effects of amendments include altering soil physical and chemical properties as well as providing substrate that enhances microbial activity.

Soil microbial communities decompose organic matter and cycle nutrients through the production of extracellular enzymes that mineralize complex compounds into inorganic molecules that can be assimilated by microbes and plants. Measuring potential extracellular enzyme activity (EEA) can therefore provide insight into the processes influencing microbial and plant growth including substrate availability, microbial nutrient demand, and overall organic matter accumulation (Sinsabaugh et al. 2008). For example, when simple N and phosphorous (P) sources were added to a system, an increase in the extracellular enzymes that target complex C sources was observed because the microbial communities became C limited relative to other nutrients (Allison and Vitousek 2005). Similarly, Bowles et al. (2014) found that C-degrading enzyme activity increased with inorganic N availability while N-degrading enzyme activity increased with C availability. Studying changes in enzyme activity due to the addition of a substrate can help to understand specific enzyme-substrate interactions and help improve current models of decomposition (Wallenstein and Weintraub 2008). Higher levels of potential EEA have been associated with greater plant productivity and nutrient mineralization, making extracellular enzymes a relevant and descriptive measure to monitor changes in soil quality and nutrient availability (Dick 1992; Bandick and Dick 1999). For example, increases in soil NO_3^- and NH_4^+ concentrations have been associated with increased N-acetyl-β-glucosaminidase activity, which degrades chitin and increases N availability (Burke et al. 2011). Since many hydrolytic enzymes are often substrate specific, measuring their activity can help identify which molecules microbes are targeting for nutrient acquisition.

The objective of this study was to examine the efficacy of biochar and woodchip amendments for enhancing plant growth and microbial community dynamics with the ultimate goal of informing plant establishment strategies in degraded dryland systems. We quantified the effects of autoclave heat shocked soils and of biochar or woodchip amendments on soil microbial biomass and activity, soil nutrient availability, and plant biomass. The autoclave heat-shock treatment is intended to mimic soil-stockpiling practices typical of mining projects in the Southwest U.S., to reduce microbial biomass and activity in soils, and to test the disruption of aboveground-belowground interactions on nutrient dynamics and plant establishment. We experimentally tested the effects of biochar, woodchip, or autoclave soil treatments on a suite of soil, microbial, and plant responses. We predicted that autoclaved soils might initially have higher nutrient concentrations than the non-autoclaved soils due to the release of nutrients stored in cellular components during cell lysis. Over time, however, we predicted a decline in nutrient availability due to the decrease in microbial community abundance and activity needed to drive nutrient transformations. We therefore hypothesized that autoclaved soils would yield lower total plant biomass with a greater proportion of biomass allocated belowground relative to aboveground compared to values observed in the non-autoclaved soils. We predicted that the high absorptive capacity of biochar would allow it to absorb and slowly release water and nutrients in the soil, which would increase availability to plants and microorganisms. This would have positive effects on plant growth and soil microbial communities (measured through enzyme activity and microbial biomass C and N). We further hypothesized that woodchip amendments would decrease soil water retention while increasing microbial biomass through immobilization of soil nutrients (e.g., dissolved organic C [DOC] and N [DON]). We predicted woodchip amendments to have a neutral to negative effect on plant biomass due to a decrease in both water and nutrient

availability. Finally, the relative abundance of DOC and DON in soils should influence microbial biomass C and N and microbial investment in C-acquiring relative to N-acquiring enzymes. We predicted that when there is greater DOC relative DON, there would be greater investment in N-acquiring enzymes relative to C-acquiring enzymes because N is more limiting.

Materials and methods

Experimental design

Soil was collected from a semiarid desert grassland in the Santa Rita Mountains approximately 60 km south of Tucson, AZ, USA (31.822370 N, 110.734166 W) on 8 Feb 2013. Soils, classified as Aridic Calciustoll (Rasmussen et al. 2015), were homogenized, sieved at 2 mm, and subdivided into three amendment treatments; unamended field soils that received no additions, biochar amended, or woodchip amended. Biochar, produced from of a mix of Northeast U.S. hardwood tree species and manufactured between 450 and 600 °C (Charcoal House LLC, Crawford, NE), was added to soil at 4% by weight following previous biochar addition experiments in semiarid soils (Artiola et al. 2012). Woodchips, harvested from *Juniperus monoserma* (Cupressaceae) trees growing on the site where soil was collected, were produced with an Electric Shipper/Shredder (Chicago Electric Power Tools, Camarillo, CA, USA). Woodchip sizes ranged from <1 mm to 21 mm. Woodchips were added at 8% by weight to standardize C addition with biochar. Common uses of woodchips in management practices include surface mulch as well as mixing woodchips into soils. In this study we examined the effects of incorporating woodchips into the soil. A third objective of the experimental design was to reduce microbial abundance while leaving physical and chemical aspects of the soil nearly unchanged, which was accomplished using an autoclave heat-shock treatment (121 °C and 15 psi for one hour; Trevors 1996; Callaway et al. 2004). This resulted in four soil treatments: field (control), autoclaved, biochar amended, and woodchip amended in a randomized complete block design with 21 replicates per soil treatment.

Soils were analyzed before seeds were planted to determine initial soil properties including: soil water content (SWC), percent organic matter (OM%), pH, bacterial cell counts (using quantitative PCR; qPCR), microbial biomass (C_{mic} and N_{mic}), and dissolved organic carbon and nitrogen (DOC and DON) (Table 1 and Table 2). SWC was measured as the difference in sample weight before and after oven drying for 24 h at 105 °C. Loss on ignition was used to determine OM% of samples by heating samples at 500 °C for 4 h (Nelson and Sommers 1965). Soil pH was measured in deionized water with a 1:2 soil-to-solution ratio using sympHony Model SB20 (Nicol et al. 2008).

We examined the effect of soil treatments on six C_4, warm-season grass species previously shown to be successful at establishing in degraded soils and that are indigenous to the site where soil was collected (Fehmi and Kong 2012). The grass species included: *Eragrostis intermedia*, *Bouteloua gracilis, Hilaria belangeri*, *Digitaria californica*, *Bouteloua curtipendula*, and *Leptochloua dubia*. Cone-tainer™ pots (20 cm depth × 4 cm diameter; Ray Leach and Sons, Tangent, OR) with 25 cm^2 mesh cloth at the bottom of each pot to prevent soil loss, were used in a growth chamber experiment. Each pot was filled with 175 g of soil of one of the six treatment combinations. Three seeds for each species were planted in monoculture in each of the six soil treatments with three replicate pots. Three unplanted control replicates were also included for each soil treatment, which resulted in 84 experimental units (six plant species and one control x four soil treatments x three replicates). Pots with no seed germination were not replanted. Pots were then brought to field moisture capacity with deionized water and placed into a growth chamber (Conviron® A1000, Conviron, Manitoba, Canada). To represent natural ambient field conditions, the growth chamber schedule was set to a day/night regime of 14 h light/10 h dark, with temperature extremes of 37 °C (day) /15 °C (night), with a maximum wind speed of 8 mph (day) and a minimum wind speed of 3 mph (night), and percent relative humidity extremes of 7% (day) / 96% (night).

Every three days, pots were watered with 8 mL of deionized water and every nine days, trays were rotated clock-wise onto different shelves in order to reduce potential effects of heterogeneous conditions within the chamber. To accommodate growth chamber dimensions, reduce light interference, and equalize light intensity among pots, all grasses across treatments were clipped to 5 cm on day 33, 50, and 69. Biomass clippings were dried at 65 °C for 48 h before weighing. On day 42, pots were weighed before and 30 min after watering, when active drainage had stopped, to determine soil water retention. After 78 days, aboveground biomass was

Table 1 Initial soil measurements. Mean values are presented ± SE (when applicable); 16S rRNA qPCR values (mean value × 10^7 ± SE value × 10^6) are presented as total number of bacterial cells / g dry soil; organic matter percentage (OM) measured by loss on ignition is presented as percent of total sample; soil water content (SWC) is recorded in grams; microbial biomass carbon (C_{mic}), microbial biomass nitrogen (N_{mic}), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) are presented as (mg / kg soil)

Treatment	16S rRNA (qPCR)	OM (%)	SWC	pH	C_{mic}	N_{mic}	DOC	DON
Field	2.98 ± 1.29	3.86	2.7	8.335 ± 0.005	0.054	0.014	0.072	0.005
Autoclaved	2.12 ± 2.74	4.15	1.6	8.265 ± 0.015	0.024	0.017	0.166	0.043
Biochar	6.24 ± 0.74	6.29	2.45 ± 0.05	8.17 ± 0.04	0.064 ± 0.008	0.015 ± 0.004	0.091 ± 0.007	0.006 ± 0.002
Woodchips	6.17 ± 1.46	7.24	2.45 ± 0.05	7.985 ± 0.005	0.288 ± 0.022	0.020 ± 0.000	0.112 ± 0.012	0.001 ± 0.000

harvested, dried as previously specified, and weighed. Belowground biomass was harvested by removing all visible roots from the soils and remaining belowground biomass was collected upon soil sieving through a 2 mm sieve. Air-dried belowground biomass was recorded. Remaining soil was air-dried, stored at 4 °C, −20 °C, or −80 °C for microbial and biogeochemical processing.

DNA extraction and quantitative PCR (qPCR)

Soil samples were stored at −80 °C prior to all DNA extraction. We used the PowerSoil® DNA Isolation kit (MoBio Laboratories) to extract DNA from 0.25 g of soil. Extracted genomic DNA was quantified using a Nanodrop spectrophotometer and samples that did not have 260 / 280 wavelength values between 1.3 and 2.3 were re-extracted. Real time quantitative PCR (qPCR) was used to estimate the abundance of bacterial cells as a measure of autoclave efficacy in the initial soil treatments.

Protocol for qPCR of the 16S rRNA gene region followed Ritalahti et al., (2006). Reactions consisted of 12.5 μL reaction volumes containing 1 μL DNA template (a 100-fold dilution), 0.5 μL of each universal bacterial primer 8F (AGAGTTTGATCCTGGCTCAG) and 1541R (AAGGAGGTGATCCAGCCGCA), 3.75 μL sterile, PCR-grade water, 0.5 μL of BAC1115Probe (5′- FAM-CAACGAGCGCAACCC-TAMRA) and 6.25 μL Quanta PerfeCTa qPCR ToughMix mastermix (Quanta Biosciences). qPCR was performed on a PikoReal™ Real-Time PCR System and PCR conditions were as follows: 95 °C for three minutes, 50 °C for two minutes, followed by 40 cycles of 95 °C for 15 s, 52 °C for 30 s, and 72 °C for 45 s. The average slope and *y*-intercept of each standard curve were determined by regression analysis and used to calculate the number of gene copies per g/soil adjusted for the dilution factor.

Table 2 DOC:DON ratios measured from soils at the onset and end of the experiment. Mean values are presented ± SE (when applicable); dissolved organic carbon (DOC) and dissolved organic nitrogen (DON)

Treatment	initial	final
Field	14.226	7.138 ± 0.581
Autoclaved	3.832	5.391 ± 0.822
Biochar	15.305 ± 3.325	5.997 ± 0.313
Woodchips	113.799 ± 30.193	24.055 ± 1.580

Potential extracellular enzyme activity

Potential extracellular enzyme activity (EEA) was measured using a fluorimetric deep-well microplate technique modified from Wallenstein et al. (2012). Prior to the assays, soil pH was used to determine the appropriate buffer solution. Soil slurries were prepared with 2.75 g of soil that was stored at 4 °C for no longer than 3 weeks and 91 mL of 50 mM Tris Buffer, which was titrated to pH 8.2. We measured potential activity of seven hydrolytic enzymes: β-D-cellubiosidase (CB), α-Glucosidase (AG), β-Glucosidase (BG), and β-Xylosidase (XYL), which hydrolyze carbon-rich substrates; leucine aminopeptidase (LAP) and N-acetyl-β-Glucosaminidase (NAG), which hydrolyze nitrogen rich substrates, and Phosphatase (PHOS), which hydrolyzes phosphorous rich substrates. Standards for standard curves and assays were incubated at 25 °C for 1.25 and 1.5 h; 100 μL of 200 μM fluorimetric substrate was added to 900 μL of each soil slurry. Fluorescence was measured on Synergy™ 4 Multi-Mode microplate reader with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Incubation time was

adjusted for samples that had activity higher than the detection limit.

Soil and microbial biomass carbon and nitrogen

Microbial biomass C and N (C_{mic} and N_{mic} respectively) in the soil was measured on a subset of the samples by chloroform fumigation extraction (Beck et al. 1997). Paired samples that were either fumigated with ethanol-free chloroform for 48 h or not fumigated were extracted with 25 mL 0.5 M K_2SO_4. Samples were shaken for 1 h, filtered, and stored at −20 °C until processing using a non-purgable-organic-C protocol on a Shimadzu total organic carbon analyzer (TOC 5000) equipped with a total dissolved nitrogen module (Shimadzu Scientific Instruments, Inc., Columbia, MD, U.S.A.). The efficiency factors for microbial biomass carbon (kEC = 0.45 [Beck et al. 1997]) and microbial biomass nitrogen (kEN = 0.54 [Brookes et al., 1985]) were used to calculate the respective biomass as the difference between fumigated and non-fumigated samples.

Soil nitrogen pools and mineralization rates

Ammonium (NH_4^+), nitrate (NO_3^-) pools, and rates of net mineralization (N-mineralization) were determined on a subset of samples using KCl extraction (Robertson et al. 1999) from 5.0 g of air dried soil that was brought up to 60% water holding capacity. Soils were incubated for a week and KCl extractions were performed on samples before the incubation period started and after seven days of incubation with 25 mL of 2 N KCl. Samples were shaken for 1 h, filtered, and stored at −20 °C until processing. All KCl extracts were analyzed colormetrically with 2-phenylphenol for NH_4^+-N (Rhine et al. 1998) and the vanadium method of Doane and Horwáth (2003) for NO_3^--N using Synergy™ 4 Multi-Mode microplate reader. Net N-mineralization was calculated as the difference between the sum of NH_4^+-N and NO_3^--N before and after the incubation.

Soil water characteristic curves

Soil water characteristic curves were generated for the field soil, and field soil mixed with woodchips or biochar following Tuller and Or (2004). Specifically, matric potential was determined using Tempe Cells for matric potentials below 300 m of head and using a Decagon WP4 water potential meter (Decagon Devices, Inc., Pullman, WA) for potentials greater than 300 m.

Statistical analysis

All data used for analysis is presented as supplemental material (Online Resource 1). All analyses were performed in R: Language for Statistical Computing 3.1.1 (R Development Core Team 2011, r-project.org). Multivariate ANOVA (MANOVA) was used to evaluate soil treatment effects on plant biomass, soil physiochemical variables, and microbial biomass and activity using the 'manova' function in the stats package. When two dependent variables had high correlations, or redundancy, with one-another ($r \geq 0.50$ or $r \leq -0.50$) only one was included in the analysis to reduce data dimensionality and improve the strength of the test statistics (Cohen 1992). Data were tested for normality using the Shapiro-Wilk test of normality ('shapiro.test' function in the stats package) and by visually examining the data. Most variables had non-normal distributions and 'box cox' transformations in the MASS package failed to normalize the data, so data were left untransformed when analyzed, unless specified otherwise. Nonparametric MANOVA ('adonis' function in the vegan package in R) on the data exhibited the same trends that parametric tests did, so parametric results are presented here since they provide more efficient inferences than non-parametric procedures. Additionally, we used Pillai as the test statistic in the MANOVA, since it is fairly robust to violations of multivariate normality (Quinn and Keough 2002). When a significant result was observed in the MANOVA, follow-up ANOVAs were performed to determine how soil treatments affected each of the measured variables (Scheiner and Gurevitch 2001). Tukey's post-hoc comparison test ('TukeyHSD' function in the stats package) was used when ANOVAs showed significance at a level of $p < 0.05$.

Principle components analysis (PCA) allowed visualization of the effects of the soil treatments on variance of the entire suite of variables measured. Variables included in the PCA included: above- and below-ground plant biomass, water retention, all seven extracellular enzyme activities, C_{mic}, N_{mic}, DOC and DON, NH_4^+, NO_3^-, and net N-mineralization. All variables were mean-centered and scaled by dividing the centered value by the standard deviation for each variable prior to analysis. This ensured that the different measurement scales and large variances

of variables did not affect the results. The 'prcomp' function in the 'stats' package was used for PCA.

Regressions were performed in R using the 'lm' function in the stats package. EEA C:N ratios were calculated as the sum of C-acquiring enzyme activities (CB, XYL, AG, and BG) divided by the sum of N-acquiring enzyme activities (LAP and NAG).

Results

The MANOVA, which combined above-ground plant biomass, water retention, BG extracellular enzyme activity, DOC, DON, C_{mic}, NH_4^+, and rates of net nitrogen mineralization indicated a significant main effect of soil treatment ($p < 0.001$, $F_{3,59} = 17.298$). Significance values for the univariate ANOVAs for each dependent variable in the soil treatments are presented in Table 3.

Table 3 Univariate ANVOA results comparing the four soil treatments (field, autoclaved, biochar, woodchips). Seventeen response variables were measured, including above and belowground biomass; soil water retention, seven potential extracellular enzyme activities, dissolved organic carbon and nitrogen (DOC and DON), microbial biomass carbon and nitrogen (C_{mic} and N_{mic}), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations, and rates of net nitrogen mineralization (N-mineralization)

	p-value	$F_{3,59}$
Aboveground biomass	6.276×10^{-3}	4.756
Belowground biomass	0.024	3.498
Water retention	0.454	0.891
β-D-cellubiosidase (CB)	3.797×10^{-3}	5.245
β-xylosidase (XYL)	2.622×10^{-4}	8.034
α-glucosidase (AG)	0.209	1.580
N-acetyl-β-glucosaminidase (NAG)	0.020	3.689
β-glucosidase (BG)	7.186×10^{-8}	19.197
Leucine aminopeptidase (LAP)	4.025×10^{-11}	34.186
Phosphatase (PHOS)	2.562×10^{-4}	8.060
DOC	$< 2 \times 10^{-16}$	76.576
DON	3.844×10^{-9}	24.406
C_{mic}	1.313×10^{-3}	6.312
N_{mic}	0.548	0.716
NH_4^+	2.241×10^{-6}	13.967
NO_3^-	2.961×10^{-6}	13.581
N-mineralization	1.828×10^{-8}	21.538

Plant biomass

Differences in aboveground and belowground biomass were significant among soil treatments (Table 3). These trends were driven by the woodchip amendment, which resulted in the lowest above- (0.006 ± 0.002 g) and undetectable belowground biomass compared to the field control (aboveground; belowground: 0.063 ± 0.010 g; 0.036 ± 0.008 g), autoclave (0.071 ± 0.022 g; 0.068 ± 0.024 g), and biochar amended treatments (0.085 ± 0.016 g; 0.061 ± 0.014 g) (Fig. 1). Differences in root:shoot ratios were also significant among soil treatments ($p = 0.017$, $F_{3,71} = 3.625$). Plants exhibited equal investment in belowground relative to aboveground growth in autoclave and woodchip amended soils (both ratios were equal to 1) while plant biomass ratios in the field and biochar amended soils exhibited the opposite trend, having more aboveground biomass in both cases (Fig. 1).

Soil physiochemical properties

Differences in DOC and DON among soil treatments were significant (Table 3). The field and autoclave treatments had similar values for both DOC (0.033 ± 0.001 mg/kg soil; 0.037 ± 0.003 mg/kg soil) and DON (0.005 ± 0.000 mg/kg soil; 0.007 ± 0.001 mg/kg soil) (Fig. 2). DOC:DON ratios were slightly higher in the field samples than in the autoclaved samples at the conclusion of the experiment (Table 2). The biochar amended had the second highest value for DOC (0.060 ± 0.003 mg/kg soil) and the highest overall value for DON (0.011 ± 0.001 mg/kg soil). The woodchip amended soils had the highest value for DOC (0.078 ± 0.003 mg/kg soil) and the lowest value for DON (0.003 ± 0.000 mg/kg soil), contributing to the highest DOC:DON ratios in these samples (Table 2).

Differences in NH_4^+ and NO_3^- concentrations were also significant among soil treatments (Table 3). The field soils had the lowest concentration of NH_4^+ (0.362 ± 0.073 μg/g soil). The three other treatments had similar concentrations of NH_4^+ ranging from 0.848 ± 0.070 μg/g soil in the autoclaved treatment to 0.645 ± 0.038 μg/g soil in the woodchip amended (Fig. 3). Average NO_3^- concentration in the field soils was 5.727 ± 0.930 μg/g soil. The biochar amended had the highest concentration of NO_3^- (10.835 ± 1.507 μg/g soil) but this was not statistically different from the autoclaved soils (9.389 ± 1.678 μg/g soil). The

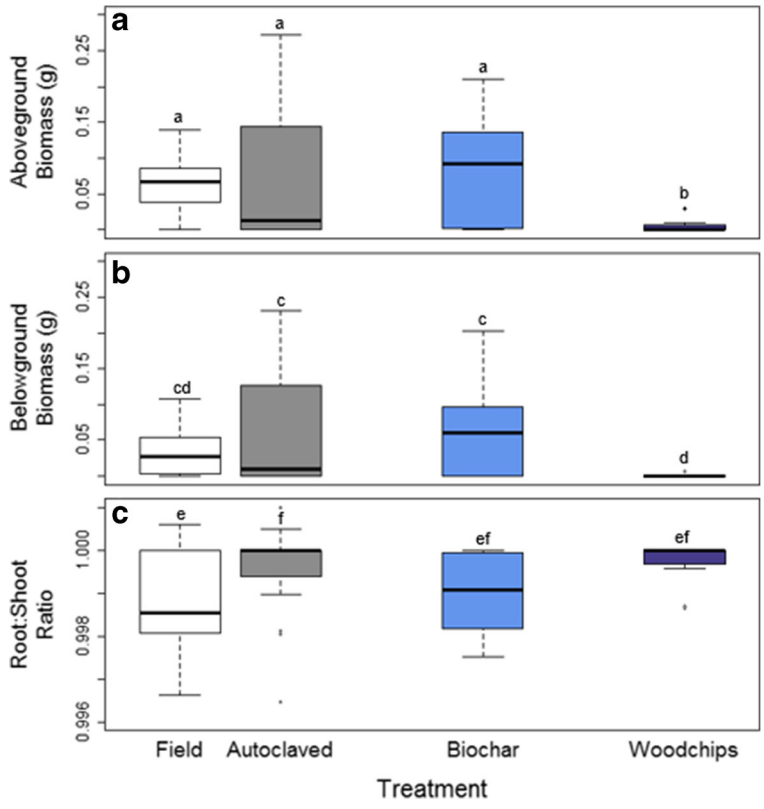

Fig. 1 Boxplots of aboveground biomass (g) **(a)**, belowground biomass (g) **(b)**, and root:shoot ratios (calculated as ln(aboveground biomass + 10) / ln(belowground biomass + 10) for each sample) **(c)** in each of the soil treatments. Solid lines across each box represent median values, lower and upper box boundaries represent the quartiles (25th and 75th respectively), bars represent the maximum (upper) and minimum (lower) values with outliers excluded, *letters* indicate TukeyHSD post hoc significance test results ($p < 0.05$) among treatments within each variable, outliers are indicated by ○, $n = 18$

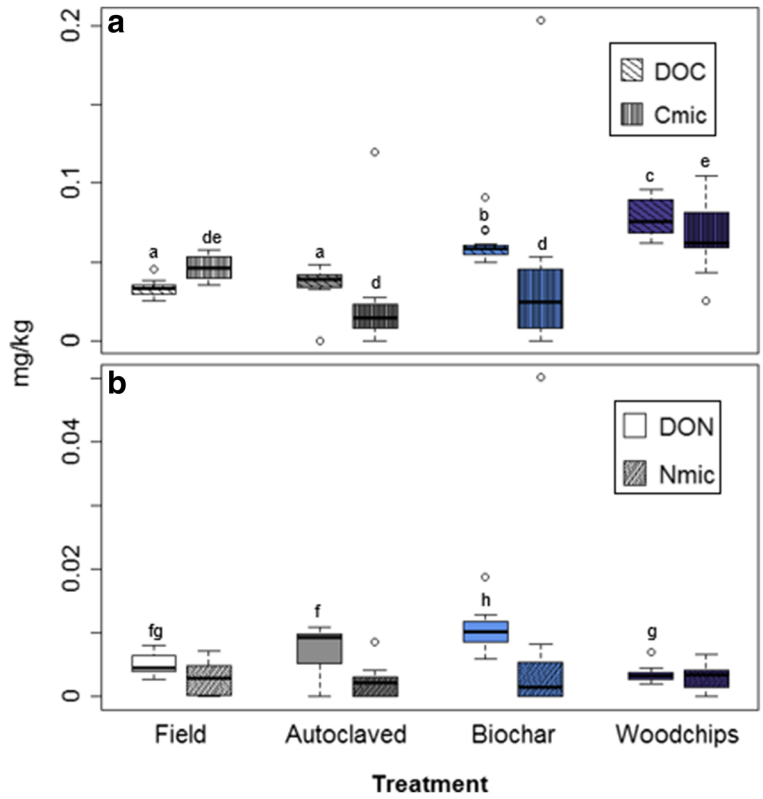

Fig. 2 Boxplots of dissolved organic carbon (DOC), microbial biomass carbon (Cmic) (mg/kg soil) **(a)**, dissolved organic nitrogen (DON), and microbial biomass nitrogen (Nmic) (mg/kg soil) **(b)** in each of the soil treatments. Solid lines across each box represent median values, lower and upper box boundaries represent the quartiles (25th and 75th respectively), bars represent the maximum (upper) and minimum (lower) values with outliers excluded, *letters* indicate TukeyHSD post hoc significance test results ($P < 0.05$) among treatments within each variable, outliers are indicated by ○, $n = 15$

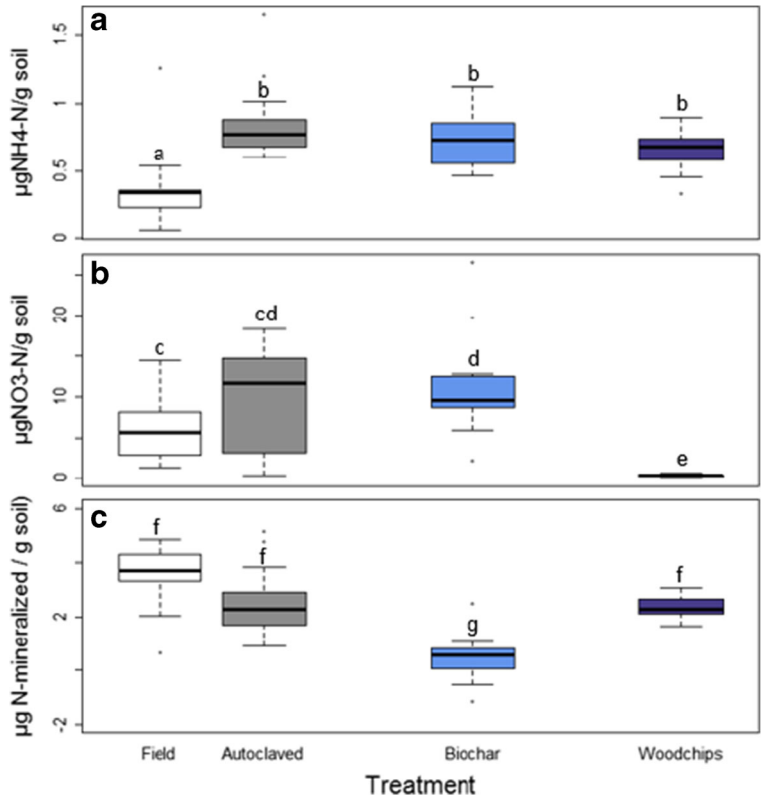

Fig. 3 Boxplots of NH_4^+ (μg NH_4^+-N / g soil) **(a)**, NO_3^- (μg NO_3^--N / g soil) **(b),** Net N-mineralization (μg N-mineralized / g soil) **(c)** in each of the soil treatments. Solid lines across each box represent median values, lower and upper box boundaries represent the quartiles (25th and 75th respectively), bars represent the maximum (upper) and minimum (lower) values with outliers excluded, *letters* indicate TukeyHSD post hoc significance test results ($P < 0.05$) among treatments within each variable, outliers are indicated by ○, $n = 15$

woodchip amended had significantly the lowest concentration of NO_3^- (0.315 ± 0.043 μg/g soil) (Fig. 3).

Both biochar and woodchips increased water holding capacity at saturation. The field soils had the lowest soil water content at saturation (31%) compared to the biochar (33%) and woodchip amended (37%) samples (Fig. 4). The biochar amended sample water content stayed close to the value observed at saturation (33%) up until field capacity (metric head = −1 m), while both the field and woodchip amended samples fell to 27% and 32% respectively (Fig. 4). Woodchip amended samples had the least amount of water available (only 6 – 7%) between field capacity and the point most plants exhibit water stress (metric head = −10 m). Biochar amended soils, on the other hand, increased plant available water shown by the 10 - 11% change in VWC between metric head of 1 and 10 m (Fig. 4).

Microbial biomass and activity

C_{mic} values were significantly different among soil treatments, while N_{mic} values were not (Table 3). C_{mic} values in the field (0.047 ± 0.002 mg/kg soil), autoclaved (0.020 ± 0.007 mg/kg soil), and biochar amended (0.037 ± 0.013 mg/kg soil) were not statistically different from one another (Fig. 2). The woodchip amended had the highest value for C_{mic} (0.068 ± 0.005 mg/kg soil) (Fig. 2).

The difference in N-mineralization was significant among soil treatments (Table 3). The biochar amended exhibited negative values for N-mineralization (−0.028 ± 0.589 μg/g soil) while the three other treatments expressed positive values ranging from (3.575 ± 0.291 μg/g soil) in the field soils to (2.352 ± 0.122 μg/g soil) in the woodchip amended soils (Fig. 3).

All extracellular enzymes assayed, with the exception of AG and NAG, varied significantly among soil treatments (Table 3). Compared to field soils, the enzyme activity in autoclaved soils was significantly reduced (Table 4). Most notably, LAP activity decreased by 70%, XYL activity decreased by 87%, and BG activity decreased by 90%. With the exception of LAP in the woodchip amended, the biochar amended soils had the highest measured activity for all enzymes (Table 4). Compared to the field soils, biochar amended soils had a 123% increase in CB activity, a 93% increase in BG activity, and an 84% increase in PHOS. The woodchip amended soils had similar activity to the field

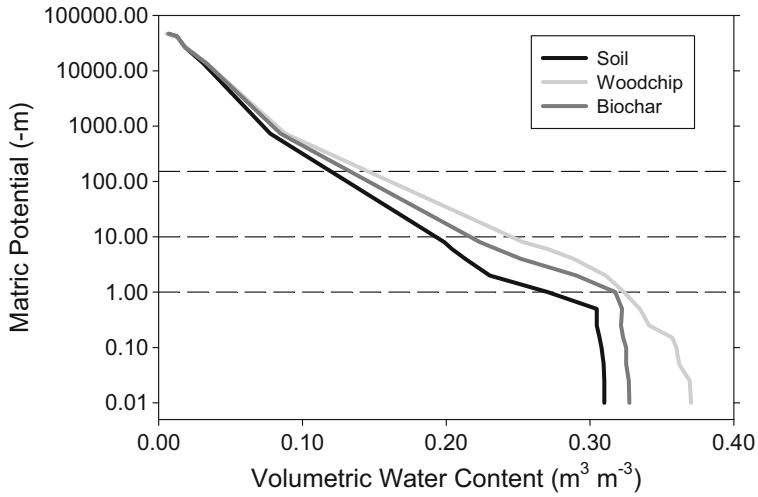

Fig. 4 Soil water retention curves determined by regressions of soil water matric potential and volumetric water content for the field (soil), biochar, and woodchip amended treatments. Dashed lines represent the permanent wilting point (150 m), the point at which many plants show moisture stress (10 m), and the moisture content at field capacity (1 m). Lines are colored according to treatments: field, biochar, and woodchips (black, gray, and light gray respectively)

soils except with LAP, where there was a 71% increase in activity (Table 4).

Principle Components Analysis

The PCA reduced 17 variables to two factors that accounted for 46.54% of the variance (Fig. 5). The loadings for PC1 were highest with all potential EEA (values ranging from −0.275 to −0.399), while the loadings for PC2 were highest with C_{mic} (0.282), DON (−0.552), NO_3^- (−0.545), and N-mineralization (0.409). Autoclaved soils had discrete separation along PC1 and the biochar amended soils had discrete separation along PC2 (Fig. 5).

Regressions

We examined the relationships between the carbon-to-nitrogen ratios in EEA, microbial biomass, and soil nutrient availability (measured as DOC and DON) (Fig. 5). There was a significant negative relationship between EEA C:N and DOC:DON ($r^2 = 0.214$, $p < 0.001$, Fig. 5a). There was a weak negative relationship between EEA C:N and C_{mic}:N_{mic} ($r^2 = 0.067$, $p = 0.046$, Fig. 5b) and a weak positive correlation between C_{mic}:N_{mic} and DOC:DON ($r^2 = 0.068$, $p = 0.044$, Fig. 5c).

Discussion

Effects of autoclave heat shock treatment

Autoclave heat shock is a commonly used method to differentiate between abiotic- and biotic-driven processes in soils (Liebich et al. 2006; Berns et al. 2008). Autoclaving soil reduces microbial community abundance and activity with minimum alterations to soil physical properties, and is therefore relevant to experiments examining soil biodegradation (Belnap 1995; Trevors 1996; Getenga et al. 2004; Berns et al. 2008). Autoclaving exposes microbial communities to extreme heat and pressure, which causes cell mortality (supported by low bacterial cell counts; Table 1) and a subsequent release of labile nutrients into soil. In our study, the predominate differences among autoclaved soil and amended or field soils related to differences in microbial biomass C, EEA, and N mineralization (Fig. 5). Similar to results from other studies (Salonius et al. 1967; Serrasolsas and Khanna 1995), NH_4^+ concentrations in this experiment were significantly higher in the autoclave treatment (Fig. 3a). In many cases, autoclaving has been shown to significantly decrease microbial activity (Stursova and Sinsabaugh 2008; Blankinship et al. 2014); although some studies have shown enzyme activity to persist. This is mostly likely due to absorption of organic matter on the active site on the enzyme, protecting it from degradation (Carter et al. 2007). Our results show that, as expected, the autoclave heat shock treatment significantly reduced microbial cell abundance and potential EEA in soil (Table 1 and 4). All seven enzymes measured in this experiment displayed potential activity in the autoclaved soils that was much lower than the potential activity observed in field soils (XYL, BG, and LAP were significantly lower; Table 4), which is expected from soils with low microbial biomass.

Table 4 Influence of soil treatments on potential extracellular enzyme activities (expressed as nmol activity h^{-1} g^{-1} SOM). Values represent means ± stderr., n = 21, *letters* indicate TukeyHSD post hoc significance test results ($P < 0.05$) among means for each of the soil treatments within each extracellular enzyme. CB = β-D-cellubiosidase, XYL = β-xylosidase, AG = α-glucosidase, NAG = N-acetyl-β-glucosaminidase, BG = β-glucosidase, LAP = leucine aminopeptidase, PHOS = phosphatase

	Field	Autoclaved	Biochar	Woodchips
CB	1.006 ± 0.164 *b*	0.384 ± 0.082 *b*	2.246 ± 0.579 *a*	1.053 ± 0.181 *b*
XYL	3.379 ± 0.508 *c*	0.433 ± 0.092 *d*	5.567 ± 1.326 *c*	3.321 ± 0.396 *c*
AG	1.207 ± 0.209 *ef*	0.635 ± 0.127 *f*	1.710 ± 0.400 *e*	1.023 ± 0.148 *ef*
NAG	1.283 ± 0.228 *gh*	0.633 ± 0.153 *h*	1.804 ± 0.349 *g*	1.886 ± 0.288 *g*
BG	9.409 ± 0.885 *j*	0.950 ± 0.171 *k*	18.204 ± 2.503 *i*	10.417 ± 0.722 *j*
LAP	36.57 ± 2.252 *m*	10.88 ± 0.850 *n*	60.79 ± 5.369 *l*	62.69 ± 3.769 *l*
PHOS	50.89 ± 5.965 *pq*	23.01 ± 3.406 *q*	93.67 ± 11.744 *o*	77.01 ± 6.578 *op*

Plants grown in autoclave heat shocked soils will interact with a depauperate microbial community that lacks many potentially symbiotic as well as pathogenic taxa that could alter plant growth and biomass allocation (Hunt and Nicholls 1986; Makoto et al. 2010; Zamani et al. 2015). In this study, microbial biomass was significantly lower in autoclaved soils, and never approached the values of field soils even after 78 days (Table 1 and Fig. 2). Furthermore, microbial biomass C:N ratios were significantly higher in field compared to autoclaved soils (Fig. 6c). This slow recovery of microbial populations could have a positive effect on plant biomass if it reflected lower C and N immobilization and therefore greater nutrient availability for plants (e.g., values of plant biomass in autoclaved soil similar to values seen in the field soils; Fig 1). Plants grown under nutrient and water limitation typically have greater root:shoot ratios with greater allocation to root biomass relative to total plant biomass (Poorter and Nagel 2000; Wang and Taub 2010; Dietzel et al. 2015). In one recent study, seedlings grown under greenhouse conditions for two weeks invested more in root biomass in the autoclaved soils than seedlings grown in unsterilized soils (Mahmood et al. 2014). Similarly, in our study, plants grown in autoclaved soils had greater root:shoot ratios compared to plants grown in field soils after 78 days (Fig. 1). Only NH_4^+ concentrations were higher in autoclaved soils, and EEA was lower for all seven enzymes tested (Fig. 2

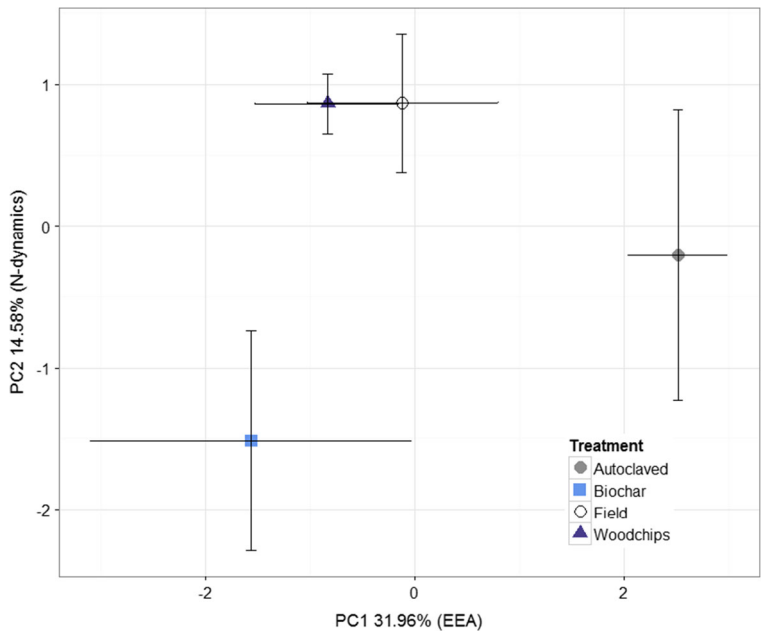

Fig. 5 Scatterplot matrix of the first two components from the PCA. Points represent average PCA values with 95% confidence intervals for each soil treatment

and Table 4). It is possible that the loss of potentially symbiotic microorganisms in the autoclaved soils could have led to increased plant investment into root biomass to forage and acquire limiting resources (Clarkson 1985; Dakora and Phillips 2002).

Effects of biochar amendments

The nutrient and water retention properties of biochar have been shown to have a positive effect on plant productivity in crop, grass, and forest ecosystems (Ohsowski et al. 2012; Biederman and Harpole 2013). Schimmelpfennig et al. (2014) found that when a grass species (*Lolium perenne*) was grown in a temperature controlled greenhouse for 5 weeks, biochar amended soils had a 29% increase in plant growth compared to the control. A study that used a similar biochar source and production condition to the biochar used in this experiment found that biochar application to dryland soils can result in positive wheat crop yields and less fertilizer requirements than soils without biochar (Blackwell et al. 2010). Our results show that plant biomass in biochar amended soils was significantly higher than in woodchip amended soils, though not significantly higher than field (control) soils (Fig 1). This could be due to the fact that plants in this experiment did not experience water limitation (Fig. 4), which is often encountered in field conditions.

Many studies have found biochar amendments increase CEC and nutrient absorption in soils, including: DOC, P, Ca, and N (Liang et al. 2006; Taghizadeh-Toosi et al. 2012; Alburquerque et al. 2014). In our study, the 0.82 and 1.20 fold increases in DOC and DON respectively suggest that the biochar released dissolved organic compounds, increasing soluble nutrient content relative to the unamended field soils (Fig. 2). Biochar produced under fast pyrolysis, which was the technique used in our study, can be a source of labile, organic C supporting microbial growth (Bruun et al. 2012). Our results are similar to studies that show the addition of biochar results in an overall decrease in N-mineralization (Deenik et al. 2010; Bruun et al. 2012; Dempster et al. 2012). The biochar amended soils did show increases in both NH_4^+ and NO_3^- concentrations at the conclusion of the experiment (Fig. 3), suggesting that rates of N-mineralization may have fluctuated throughout the experiment. While DOC and DON increases demonstrated enhanced nutrient availability in our study, trends in microbial biomass and measured activity suggest complex interactions among soil treatment, amendments, and plant communities.

Biochar produced from lignin-rich substrates can have rapid, positive effects on microbial abundance (Gul et al. 2015). In our study, which is similar in duration and lignin-rich biochar content to Gul et al. (2015), initial microbial abundance in the biochar amended soils was more than double the values observed in the unamended soils (Table 1). Although final values of microbial biomass were not significantly different in the biochar amended soils, N_{mic} doubled (Fig. 2). Microorganisms can enhance the cycling of stable N following biochar application, which can cause high C:N ratios in the soil similar to ones seen here (Fig. 2; Tian et al. 2016). Güereña et al. (2013) observed that soils amended with biochar had an 82% reduction in total N measured in leachate and a 37% increase in N retention in a maize cropping system. Most of this N retention was seen in soil microbial biomass, which increased three-fold (Güereña et al. 2013). It is also possible that the biochar served as a source of C, which altered C:N ratios in the soil and resulted in overall immobilization of inorganic N into microbial biomass (Fig. 2; Bruun et al. 2012).

Potential extracellular activity in soils reflects nutrient availability as well as stoichiometric demands of microbes and plants (Bell et al. 2014). As expected, the stoichiometry of EEA was negatively correlated with soil DOC:DON as well as microbial biomass C:N stoichiometry (Fig. 6a, b). Microbial EEA C:N ratios were always less than one, perhaps implying there was more activity directed towards acquiring N relative to C in these dryland soils regardless of amendment or treatment (Fig. 6a, b). Potential EEA in biochar amended soils was significantly different from that of all other soils (Fig. 5). This could be explained by biochar enhancing microbial growth, and/or causing shifts in community composition (Lehmann et al. 2011) if the microbial communities in the biochar amended soils were producing certain enzymes to acquire proteins and amino acids needed for growth and survival (e.g., PHOS, LAP; Table 4). The increase in LAP activity might also help explain the high NH_4 and NO_3 values (Fig. 3). As the DOC:DON ratio increased from one, and more organic C was present in the soil relative to N, the potential activity of N mineralizing enzymes increased (Fig. 6a). The response of EEA to biochar amendments differed from that of woodchip or no amendments (Fig. 5, Table 4), and highlights the influence of these

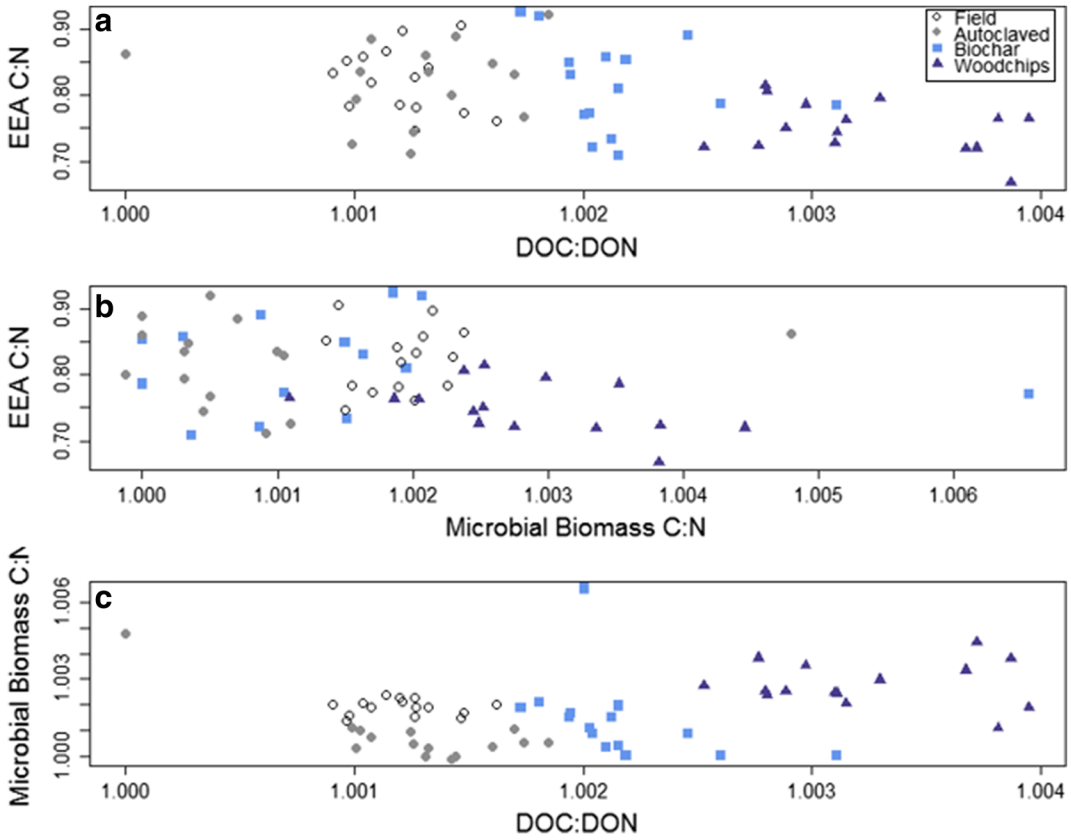

Fig. 6 Relationships between EEA C:N (calculated as the sum of CB, XYL, AG, and BG activity divided by the sum of LAP and NAG activity) and DOC:DON **(a)**, EEA C:N and C_{mic}:N_{mic} **(b)**, and C_{mic}:N_{mic} and DOC:DON **(c)**. Points are symbolized according to treatments: field, autoclaved, biochar, and woodchips (hollow circles, solid circles, solid squares, and solid triangles respectively), all variables were log transformed after a constant of 10 was added

different amendment types on soil properties such as water retention and dominant substrate forms that influence the measured enzyme activity (Bailey et al. 2011).

Effects of woodchip amendments

Although woodchips can increase water infiltration, reduce evaporation from soil, and serve as a potential nutrient source for plants (Stratton and Rechcigl 1998), impacts on plant growth and microbial dynamics are not well represented in the literature, especially from semi-arid systems. There is evidence from a limited number of studies that woodchip amendments reduce plant establishment and growth. When woodchip amendments are used after forest thinning, reductions in plant richness, diversity, and plant-cover compared to non-amended plots can persist over three growing seasons (Miller and Seastedt 2009). Our results also show lowest plant biomass in the woodchip amended pots; aboveground biomass was 90% lower than in the unamended pots and belowground biomass was nearly undetectable (Fig. 1). Nutrient or water deficiencies are likely the primary causes of poor plant growth in woodchip-amended soils. Although woodchips increased water holding capacity at saturation, the extra water drained quickly with minimal increase in potential, and contained less available water between field capacity and the point most plants exhibit water stress than the field or biochar amended samples (Fig. 4). Although DOC values increased in the woodchip-amended soils in our study (Table 1 and Fig. 2), it is possible that some of this increase was due to aromatic carbon compounds present in the juniper mulch. Furthermore, woodchip amendments lower bulk density of soils, which can limit relative nutrient availability compared to unamended field soil (Sanchez et al. 2009). For example, seedlings of *Betula papyrifera* sown in woodchip amended pots grew significantly larger with fertilizer addition (Venner et al. 2011). Plants in our study may have been N-limited, as seen by the overall decrease in inorganic N

values in the woodchip amended pots and very high initial DOC:DON ratios (Table 2 and Fig. 3a-b). As such, woodchip amendments are often combined with fertilizer treatments due to concerns regarding nutrient limitation.

Due to the high C:N ratio in woodchips, N-immobilization can occur as woodchips decay, often causing depletions in available soil N (Stratton and Rechcigl 1998; Van Rensburg and Morgenthal 2004; Homyak et al. 2008; Rhoades et al. 2012). A number of studies have looked at the potential woodchip amendments offer in immobilizing N, especially NO_3^-, for the protection of ground water (Homyak et al. 2008; Moorman et al. 2010; Li et al. 2013). Woodchip amendments have an extremely high potential to reduce NO_3^- leaching by immobilizing large amounts of N in soil (between 19 and 38 kg N ha^{-1} in one year; Homyak et al. 2008). Similar to results seen in our experiment, woodchip amendments can result in decreased NO_3^- (Fig. 3b) (Greenan et al. 2006; Homyak et al. 2008; Moorman et al. 2010). We expected N-immobilization to be reflected in N_{mic} values, but woodchip amended soils were not significantly different from field soils (Fig. 2b, Fig. 5) and had higher microbial biomass C relative to N (Fig. 6c). However, C_{mic} values decreased by 76% over time, implying that much of the microbial biomass was lost in the woodchip amended soils throughout the duration of the experiment (Table 1 and Fig. 2a). Although woodchip amendments can reduce soil available N, this effect can diminish overtime (Rhoades et al. (2012). In our study, DOC:DON ratios were 700% higher than the field soils at the onset of the experiment, but this ratio decreased by 79% (Table 2), indicating altered abiotic conditions and increasing biotic activity.

The high C:N ratios in woodchips should have strong effects on C and N microbial activity. Woodchip amended soils had the lowest potential activity of C degrading enzymes relative to N mineralizing enzymes compared to the other treatments (Fig. 6a, b). In fact, the N mineralizing enzyme, LAP, had significantly higher potential activity in the woodchip amended soils (Table 4). This increase might explain why N-mineralization rates were high even though NO_3^- values were low, as well as the dramatic decrease in DOC:DON ratios over time (Table 2 and Fig. 3). Woodchip amendments have been shown to increase both cellulose activity and fungal species abundance in an avocado orchard (Downer et al. 2002), and there is broad evidence that fungal communities shift in response to carbon sources (Hanson et al. 2008). No C-degrading enzymes studied here exhibited a significant increase in activity when woodchip amendments were applied (Table 4). Although woodchips can act as an organic C source due to their high C:N ratios, seen by the increase in DOC values here (Fig. 2a; see also (Stratton and Rechcigl 1998)) much of this carbon is bound in complex polymers. It is possible that as simple sugars were consumed in the woodchip treatments and carbon was incorporated into microbial biomass (Fig. 2a), these soils may have lacked the adequate microbial communities needed to mineralize more complex polymers, such as plant lignin, which are primarily broken down by fungal species in a multi-enzymatic process (Leonowicz et al. 1999; Chapin et al. 2011). Thus, differences in microbial community and functional diversity will likely yield different responses to woodchip amendments.

Conclusions

We compared the effects of biochar and woodchip amendments as well as soil degradation (using autoclave heat shock) on soil microbial biomass and activity, soil nutrient availability, and plant biomass. In this study, soil degradation and amendments, via changes in soil water retention and nutrient availability, had a direct effect on plant establishment and above- and belowground biomass allocation, soil physiochemical properties, and soil microbial activity. Importantly, biochar and woodchip amendments had dramatically different effects on plant biomass. Our results suggest biochar amendments can improve soil quality and promote above- and belowground plant biomass while woodchip amendments suppress plant biomass. Biochar and woodchip-amended, as well as autoclave heat shocked soils all promoted greater root:shoot ratios than field (control) soils. We found that the autoclave treatment resulted in a decrease of microbial activity while the addition of amendments caused changes in activity that could be partially explained by changes in nutrient availability. Across all soil treatments, the stoichiometry of potential extracellular activity in soils was negatively correlated with microbial biomass C:N as well as DOC:DON stoichiometry. These results reflect a response to soil nutrient availability, as modified by autoclaving or biochar and woodchip amendments as well as stoichiometric demands of microbes and plants.

Microbial biomass and EEA in autoclaved soils did not recover to values of field soils in this 78-day experiment. Furthermore, amendment additions increased carbon, nitrogen, and phosphorus mineralizing enzyme activities with no significant change in microbial biomass indicating that soil recovery in dryland ecosystems is a potentially long-term process. Better understanding of plant and microbial recovery responses to soil degradation and commercially viable amendments can help to improve the efficiency of native plant revegetation in these vulnerable dryland ecosystems.

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