# SOIL AMENDMENT EFFECTS ON DEGRADED SOILS AND CONSEQUENCES FOR PLANT GROWTH AND SOIL MICROBIAL COMMUNITIES

by

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# EXPERIMENTAL MANUPULATION OF SEMI-ARID SOILS AND THE EFFECTS ON SOIL PHYSICAL AND BIOTIC PROPERTIES AND PLANT GROWTH AND ESTABLISHMENT

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**Abstract**

Human activities that disrupt soil properties are fundamentally changing ecosystems. Soil degradation decreases microbial abundance and activity, leading to changes in nutrient availability, soil organic matter, and plant growth and establishment. Land use and land cover change are widespread and increasing in semiarid regions of the southwestern US, which results in reductions of native plant and microbial abundance and community diversity. Here we studied the effects of soil degradation and amendments (biochar and woodchips) on microbial activity, soil carbon and nitrogen availability, and plant growth of ten semi-arid plants species native to the southwestern US. Results show that woodchip amendments result in poor overall plant growth, while biochar amended soils promoted plant growth when soil quality was reduced. Additionally, amendments had a strong influence on microbial activity, while the presence and species identity of plants did not. Biochar amended soils led to increases in the potential activities of enzymes involved in the degradation of carbon, nitrogen, and phosphorus rich substrates. Woodchips, caused an increase of potential activity in enzymes involved in the degradation of sugar and proteins. These results show that microbes and plants respond differently to soil treatments and suggest that microbial responses may function as earlier indicators of the success of re-vegetation attempts.

## 1. Introduction

Understanding the strengths and directions of feedbacks between belowground and aboveground processes in semi-arid ecosystems is critically important to mitigating ecosystem

disturbance and retaining or reestablishing native plant communities. Arid and semi-arid ecosystems account for approximately one-third of terrestrial land surface area and consequently play a significant role in global water and biogeochemical cycles (Asner et al., 2003; Reynolds et al., 2007). In the Southwest U.S., land use change and non-native plant invasions are widespread and increasing (Skole et al., 1997; Williams and Baruch, 2000), typically resulting in losses of native plant biodiversity that have the potential to cascade to changes in microbial community structure and function (Wardle, 2006). Furthermore, projected warmer and drier conditions in this region have the potential to disrupt the function and stability of ecosystems and soil resources through direct effects on soil microbiota and plant-microbe interactions. A key unknown is microbial mediation of plant-soil interactions, which we investigate here via soil amendment manipulations to educate plant management strategies.

Plant communities in the Southwest U.S. have been changing dramatically over the last 100 years (Harris, 1966). Our study focuses on soils taken from the Santa Rita Mountains, located 31 km southwest of Tucson, AZ. Historically, grasses dominated these semiarid systems. Over centuries, the introduction of woody shrubs and forbs, their encroachment, and the proliferation of non-native invasive grasses has changed ecosystem processes. This has occurred through reductions in native plant diversity, alterations in resource availability, and a transformation in the fire regime (Williams and Baruch, 2000). Certain plant functional types, such as woody shrubs, have been shown to increase the competitive advantage of other functional types, like grasses, when they are present together through alterations on water availability, nutrient contents, and carbon assimilation rates and growth (Armas and Pugnaire, 2005). Plant communities in the Southwest are currently facing many threats, including land use change, cattle grazing, mining, and projected warmer and drier climate conditions (Ellison, 1960; Harris, 1966; Swetnam and Betancourt, 1997), which may fundamentally alter their composition, rates of primary productivity, and nutrient cycling.

Soil microbiota, including archaea, bacteria, and fungi, drive biogeochemical cycles that maintain soil fertility (Morris and Blackwood, 2007) and structure plant communities via positive and negative interactions (Bever, 2003). For example, plant-microbe interactions may include positive feedbacks where changes in communities of arbuscular mycorrhizal fungi (AMF) may favor certain plant species over others (Vogelsang and Bever, 2009). Microbial community diversity, structure, and consequently function respond quickly to disturbances. These changes affect plant species competitive abilities and distributions by effects on plant growth, seed production, and chlorophyll content (a trait shown to be correlated with nutrient availability) (Lau and Lennon, 2011). Soil bacterial communities have been shown to vary significantly with plant type in hot, arid systems of the Sonoran Desert (White et al., 2009). Changes in soil microbial communities might be used as indicators of changes in ecosystem resilience and impending vegetative shifts. However, specific mechanisms leading to functional similarities and differences among soil biotic communities remain poorly understood (Fierer et al., 2009). Measuring the activity of these communities, via extracellular enzyme assays, can provide insight on these mechanisms including substrate availability, microbial nutrient demand, soil pH, and overall organic matter accumulation (Sinsabaugh et al., 2008). Identifying key microbial functional groups that are associated with native grasses, forbs, and shrubs will potentially offer key insights into the ecology of these species.

Re-vegetation attempts of disturbed lands can be improved upon by considering soil microbial communities in the process. Certain plant functional types or species have been shown to have strong associations with particular microbes in soils. For example, when nutrients or protection is required by host plants, nitrogen fixing bacteria and mycorrhizal fungi, present in the soil, have been shown provide these needs (Harrison, 1999; Reynolds et al., 2003). If wanting to re-establish or grow these host plants, soil microbes are an important factor when considering attributes of soil quality (Brockway and Outcalt, 2000). Plants have been shown to be 15% more successful at establishing in semiarid environments

when particular soil microbial symbionts are present (Requena et al., 2001). At the same time, soil microbial communities are highly responsive to losses or additions of plant species (Bever et al., 2010; Goddard and Bradford, 2003) and shifts from bacterial to fungal populations are known to accompany shifts from herbaceous to woody plant dominated landscapes (Aanderud et al., 2008; Imberger and Chiu, 2001; Purohit et al., 2002). Examples from invasive plant studies suggest that soil-microbial legacies created by non-native invasive plants either directly reduce or inhibit native plant growth (Rudgers and Orr, 2009; Vogelsang and Bever, 2009), and the rates of microbial turnover and recovery remain unknown. Understanding how the structure and function of soil microbial communities driving nutrient cycling can alter, limit, or promote plant growth and in turn how plants affect microbial communities will improve our understanding of the ecology of Southwest semi-arid ecosystems and offer potential for maintaining or reestablishing native plant communities.

Another common way to enhance plant establishment in degraded soils is through the use of soil amendments. Degraded soils often contain lower organic matter, nutrients (including plant available ammonium, nitrate, phosphorus, and potassium), and microbial activity than undisturbed healthy soils, which results in less successful plant establishment (Garcia et al., 1994; Wong, 2003). The addition of amendments to degraded soils could improve deficiencies in nutrients and organic matter, alter soil porosity, and increase microbial biomass to improve plant establishment (Ehaliotis et al., 1998; Sohi, 2010; Steinbeiss et al., 2009). There are a wide variety of organic materials, including woodchips and biochar (created through the pyrolysis of plant biomass) that can be used as amendments at relatively low costs. Biochar is becoming increasingly recognized as a popular option for soil amendments. The use of biochar has two simultaneous, positive effects- mitigate anthropogenic effects of climate change by incorporating biochar into soils while improving soil fertility and plant growth (Atkinson et al., 2010; Laird, 2008). Wood-based biochars have been shown to have extremely high carbon (C) to nitrogen (N) and carbon to phosphorous (P) ratios (Kookana et al., 2011). Additionally, the high surface area of

biochars can cause absorption of water and nutrients in the soil followed by a slow release of these resources over time (Artiola et al., 2012). Woodchips reduce erosion and runoff in soils by forming mini-channels, which can trap water and sediment (Foltz and Copeland, 2009). They have also been shown to alter porosity when incorporated into soils and change nutrient dynamics by increasing N availability to plants (Miller and Seastedt, 2009). We examined both amendments to determine their effects on soil nutrient availability, plant growth, and microbial activity in soils from the Southwest U.S.

Since soil microbial communities have such an impact on vegetation, forming restoration plans that incorporate microbial communities would result in more successful plant establishment. Plant diversity and cover along with soil microbial community abundance and diversity have both been identified as important factors in restoration planning (Aronson et al., 1993). Plant diversity can help a system combat potential stressors and remain stable (Frank and McNaughton, 1991) and it can have dramatic effects on soil microbial communities and the processes they mediate including microbial biomass, respiration, and N-mineralization rates (Zak et al., 2003). Soil microbial communities influence factors that are important to consider in restoration planning including biogeochemical cycles, soil structure, and plant-soil interactions (Harris, 2009). By understanding the size, potential activity, and functional composition of soil communities we can predict the effects management plans might have on the system (Harris, 2003). Although many plant-soil interactions are context dependent, the development of successful re-vegetation plans relies on incorporating the existing knowledge we have of plants, soil microbial communities, and their interactions with one another to develop future recommendations (Eviner and Hawkes, 2008).

The objective of this study was to test feedbacks between plant functional groups and microbial functional groups in healthy and degraded soils in order to identify strategies for improving soil quality and plant establishment. To mimic the state of extremely degraded soils, which in turn allowed us to study the effects on plant establishment and growth, we used autoclave sterilization to reduce microbial

populations from field-collected soils (Wolf et al., 1989). Amendments of biochar and woodchips to field and sterile soils were used to examine interactive effects on microbial community recovery and plant establishment. Four plant functional groups, including warm-season perennial grasses, a cool-season perennial grass, an annual forb, a perennial forb (broadleaved flowering plant), a shrub, and mixed communities of these groups (Table 1), were planted to examine the effects of specific plant-microbe feedbacks. We predict that degraded soils will have a reduction in quality compared to healthy soils, which will be reflected by a decrease in microbial abundance, activity, nutrient use efficiency, and plant growth. Amendments will alter nutrient dynamics, which will cause changes in microbial activity and result in more plant growth when this shit favors the plants. Autoclave heat treatment will kill many microbial cells, thereby causing a decrease in microbial activity. A reduction in activity will cause microbes to be less efficient at making nutrients available that plants need for growth. This will cause lower overall plant growth in the degraded soils. Amendments will provide more nutrients to the soil through sorption or decomposition. If amendments make certain nutrients available in excess, microbes will shift their activities to alternate nutrient cycles. Plant growth will be greater in amended soils if amendments and microbes are successful at making nutrients plants need available.

## 2. Methods

### 2.1 Experimental set-up and design

Soil was collected from the Santa Rita Mountains approx 65 km south of Tucson, AZ, USA (31.822370N, 110.734166W) in spring 2013. Soils were either left un-amended, or were amended with biochar or woodchip addition. Biochar and woodchip amendments were added to the soil at 4% and 8% by weight respectively, following previous biochar addition experiments in semiarid soils (Artiola et al., 2012). Biochar was produced from a mix of Northeast U.S. hardwood tree species by Charcoal House LLC, Crawford, NE. Woodchips were generated from *Juniperus monosperma* trees growing on the site where the soil was collected. Half of the soil from each of the three amendment treatments (biochar,

woodchip, or no amendment) was autoclaved at 270˚C and 28psi for one hour, resulting in six soil treatments (Fig. 1). The experiment was set up as a randomized complete block design with three replicates per soil treatment.

Ten individual plant species and four community mixtures were studied (Table 1). Our focal plant species are native inhabitants of these Southwest U.S. grasslands and have been previously studied and shown to have successful growth in this area (Fehmi and Kong, 2012). We tested a range of plant functional types including: warm-season perennial grasses, a cool-season perennial grass, an annual forb, a perennial forb (broadleaved flowering plant), and a shrub. Grasses were all members of the plant family Poaceae. This group was further delineated into warm season C4 grasses and cool season C3 grasses based on their seasonality. Two common forbs in the Papaveraceae and Asteraceae and a common nitrogen-fixing shrub in the Fabaceae were also studied (Table 1).

Cone-tainer™ pots (Stuewe and Sons Inc, Tangent, OR) used in the growth chamber experiment were approximately 20cm tall with a diameter of approximately 4 cm. To prevent soil loss, 25 $cm^2$ mesh cloth was placed at the bottom of each pot. Each pot was filled with approximately 175 g of soil of one of the six treatment combinations. Three seeds were planted in each of the pots- three of one species for the individual pots and one seed of each of the three species in the community pots (Table 1). Since we wanted to study the effects of soil treatments on plant growth and establishment, we did not replant pots that had no seed germination. Three unplanted control replicates were included for each soil treatment. This resulted in 270 pots: 6 soil treatments x (10 individual species + 4 communities + 1 control) x 3 replicates. Pots were then watered to field capacity with distilled water (DI) 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 14h light/10h 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 8mL of DI water and plant heights were recorded. Every nine days, trays were rotated clock-wise onto different shelves in order to reduce potential effects of heterogeneous conditions within the chamber. As time progressed, some of the grasses needed to be clipped because they were approaching their maximum height allotment inside the chamber. All grasses over 5cm were clipped at three different time points throughout the experiment on day 33, 50, and 69. Biomass clippings were dried at 65˚C for 48hrs, then weighed. On day 42, the weights of each pot were measured before watering and 30 minutes after watering to determine water drainage values. Drainage values were measured as difference in weight immediately after watering and 30 minutes later such that, higher values represent more water drained (or lost) from the system and lower values represent less drainage.

Final plant heights were recorded after 78 days. Aboveground biomass was 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 1.981mm sieve. Fresh weight of the belowground biomass was recorded. Roots were then stored in a 70% ethanol solution for preservation. Soil that passed through a 1.981mm sieve was stored at appropriate conditions (e.g., air-dried, stored at 4˚C, -20˚C, or -80˚C) for microbial and biogeochemical processing.

**2.2 Soil Characterization**

Organic matter percentage (OM%) was measured by weight loss after ignition (Nelson and Sommers, 1982) on soils after amendments were added and autoclave heat treatment occurred.

**2.3 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 measured

in DI water with a 1:2 soil-to-solution ratio using a sympHony Model SB20 (Nicol et al., 2008) to determine the appropriate buffer solution for each soil slurry. Briefly, our soil slurries were prepared with 2.75g of soil that was stored at 4˚C for no longer than 3 weeks and 91mL of fresh 50mM Tris Buffer, which had its pH adjusted to 8.2 with concentrated hydrochloric acid (HCl). We measured potential activity of seven 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 substrate. The standards used to calibrate the assays ranged from 0μM-25μM due to the high activity observed in some samples. 7-amino-4-methylcoumarin (MUC) was used to generate the standard curve for LAP and 4-methylumbelliferone (MUB) was used as the standard for all other enzymes assayed.

Standards were incubated at 25˚C for 1.25hrs. 100μL of 200μM fluorimetric substrate was added to 900μL of each soil slurry. Assays were conducted at 25˚C and 35˚C for 1.5hr and 0.75hr respectively. Fluorescence was measured on Synergy™ 4 Multi-Mode microplate reader with an excitation wavelength of 365nm and an emission wavelength of 450nm. Incubation time was adjusted for samples that had activity higher than the detection limit.

**2.4 DNA extraction and qPCR**

Soil samples were stored at -80˚C prior to all DNA processing. We used the MoBio PowerSoil® kit to extract DNA from 0.25g of soil. Extracted genomic DNA was quantified using a Nanodrop spectrophotometer before any additional downstream processing. All samples that did not have 260/280 wavelength values between 1.3 and 2.3 were re-extracted, ensuring that only quality samples were further processed. Quantitative PCR (qPCR) was used to estimate the abundance of bacterial cells in the initial soil treatments.

Quantative PCR (qPCR) protocol followed Ritalahti et al., 2006. Briefly, qPCR assays consisted of 12.5µL reaction volumes containing 1µL DNA template, 0.5µL of each primer, 3.75µL sterile, PCR-grade water, 0.5µL probe, and 6.25µL Quanta PerfeCTa qPCR ToughMix mastermix. Genes were amplified using universal bacterial primers 8F and 1541R. Probes used were 6 FAM/TAM. PCR cycle parameters were 95°C for three minutes (denaturation), 50°C for two minutes, 95°C for three minutes, cycle 40 x 15 seconds at 95°C, 52°C for one minute, then hold at 60°C.

**2.5 Soil microbial carbon and nitrogen extraction**

Microbial biomass carbon and nitrogen in the soil was measured through the chloroform fumigation extraction method (Beck et al., 1997). Paired 5.0g sample that were either fumigated or not with ethanol-free chloroform for 48hrs were extracted with 25mL 0.5M $K_2SO_4$. Samples were shaken for 1h, 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 and Landman, 1985)) were used to calculate the respective biomasses.

**2.6 Soil nitrogen pools and mineralization rates**

Ammonium, nitrate pools, and rates of net mineralization were determined using KCl extraction (Robertson et al., 1999) from 5.0g of air dried soil that was brought up to 60% water holding capacity. Paired samples were either incubated or not for seven days and extracted with 25mL of 2N KCl. Samples were shaken for 1h, filtered, and stored at -20°C until processing. All KCl extracts were analyzed colormetrically for $NH_4^+$-N and $NO_3^-$-N using Synergy™ 4 Multi-Mode microplate reader(Doane and Horwáth, 2003; Rhine et al., 1998). Net N mineralization was calculated as the difference between the sum of $NH_4^+$-N and $NO_3^-$-N before and after the incubation.

### 2.7 Statistics

All statistical analysis was performed using R software v.3.1.1. To estimate the effects of plant functional groups, amendments, or sterilization, a 3-way ANOVA was performed. When the 3-way interaction was not significant ($p<0.01$), a 2-way ANOVA was performed with amendments and sterilization as independent variables. To determine whether plant species or plant functional groups had an effect on potential extracellular enzyme activity, all pots with total above- and belowground biomass equal to zero were removed from analysis. These samples were included when looking at the amendment and sterilization interactions. Significant differences for the soil treatments were determined using Tukey post-hoc comparison test.

For cluster and principle component analyses, variables analyzed included: above- and belowground biomass, drainage, $NH_4^+$, $NO_3^-$, net N-mineralization, and potential extracellular enzyme activity. Samples with missing data points for one or more of these variables were excluded from analysis. All variables were standardized by subtracting the mean and dividing by the standard deviation prior to analysis, which ensured the different scales variables were measured on and the large variances of variables did not have an effect on the results.

Cluster analysis is an exploratory data analysis technique that enables grouping of data points such that object belonging to the same group resemble each other in same way while objects in different groups are dissimilar. For the cluster analysis, Euclidean distance was used to calculate the dissimilarity matrix from the standardized variables and unweighted paired group means analysis (UPGMA) was used as the clustering method to create the dendrograms.

## 3. Results

### 3.1 Effects of treatments on soil properties

Table 2 shows the results of amendment and sterilization on percent organic matter (OM%), soil water content (SWC), pH, microbial biomass C and N, dissolved organic C (DOC), total N (TN) and soil

drainage. Percent organic matter (OM%) was highest in the woodchip-amended soils, and in both amended treatments, OM% was lower in the sterilized soils. A reverse trend was observed in the non-amended treatment. Microbial biomass C and N at the beginning of the experiment was also highest in the woodchip-amended soils. Sterilization reduced microbial biomass C values relative to the field soils. Sterilized soils amended with biochar had the lowest microbial biomass C values. In the non-amended and biochar-amended soils, microbial biomass N was higher in the sterilized treatments, while it was higher in the field soils amended with woodchips. The addition of woodchips lowered pH values from 8.30 ± 0.02 to 7.79 ± 0.10.

Soil water content (SWC) was lower in all sterilized treatments when compared to the field counterparts (Table 2). Sterilized soils amended with woodchips had the lowest SWC. Similarly, drainage was highest in this treatment. Both non-amended and woodchip-amended soils had more drainage in the sterilized pots compared to the field counterparts. This trend was reversed in the biochar-amended pots where drainage was higher in the field soils.

Autoclave sterilization decreased bacterial cell counts, measured by qPCR, in all soils. Bacterial cell abundance was lowest in the sterilized soils amended with woodchips and highest in field soils amended with biochar (Table2).

Net N-mineralization was significantly lower in the biochar-amended soils (Figure 3). In both the non-amended and woodchip-amended soils, sterilization slightly decreased net N-mineralization. In the biochar-amended soils sterilization slightly increased net N-mineralization.

**3.2 Direct effects of soil treatment and amendments on plant growth**

We found that the sterilization and amendment interaction had a significant effect on both aboveground and belowground plant growth (2-way sterilization:amendment ANOVA, aboveground biomass $F_{2,174}$=2.692 p=0.0706; 2-way sterilization:amendment ANOVA, belowground biomass $F_{2,174}$=3.167 p=0.0446). In the biochar-amended soils, both aboveground and belowground plant

biomass were higher in the sterilized treatment, while this trend was reversed in the woodchip amended soils (Figure 2). This was true for all plant functional types except the forbs, *E. Mexicana* and *B. multiradiata*, where the field soils amended with biochar had more belowground biomass than the sterilized soils with biochar.

Sterilization alone had a significant effect on belowground plant biomass (1-way sterilization ANOVA for belowground biomass $F_{1,174}$=5.022, p=0.0263). In both the non-amended and biochar amended soils, the sterilized treatments had more belowground plant biomass than the unsterilized treatments. This trend was reversed in the woodchip-amended treatments.

We also found that amendments alone had a significant effect on aboveground and belowground plant biomass (1-way amendment ANOVA, aboveground biomass $F_{2,174}$=16.081, p<0.01; 1-way amendments ANOVA, belowground biomass $F_{2,174}$=14.155, p<0.01). The biochar-amended soils had the highest overall plant growth, while the woodchip amended soils had the lowest. This was true in all plant functional types except the forbs, *E. Mexicana* and *B. multiradiata*, where the non-amended treatments resulted in more growth than the biochar-amended soils.

**3.3 Direct effects of soil treatment and amendments on enzyme activity**

The interaction of sterilization and soil amendments on potential extracellular enzyme activity was significant for all enzymes tested, except NAG (ANOVA p-values < 0.01; Table 4). Non-sterilized field soils amended with biochar resulted in the highest potential EEA relative to all other treatments. In the case of LAP, potential activity in the field soils amended with woodchips were not significantly different from field soils amended with biochar (Table 3). With the sterilized soils, LAP had the highest potential activity (24.08±1.09) in the woodchip-amended soils.

Three of the four enzymes involved in the degradation of carbon-rich substrates (XYL, AG, and BG) showed an increase in potential activity in the sterilized soils amended with woodchips relative to the non-amended soils (Table 3). Potential phosphatase activity was similar in sterilized soils treated

with both amendments, and higher than the sterilized soils with no amendments. This trend was also observed in the field soils. Sterilization resulted in significantly lower potential EEA relative to the non-sterilized field soils (Table 4). Two of the carbon degrading enzymes- CB and XYL- showed the largest decrease in activity when sterilized.

Amendments had a significant effect on potential EEA of CB, XYL, BG, LAP, and PHOS (Table 4). Biochar caused an increase in the potential activity of all of these enzymes. Woodchips also caused an increase in BG, LAP, and PHOS and an overall decrease in CB and XYL. LAP was highest in the woodchip treatments. NAG values were higher in both of the amended soils relative to the non-amended soils but these values were not significantly different from each other. AG values were similar across amendments.

**3.4 Feedbacks between plant-microbe interactions in response to soil treatments and amendments**

A cluster analysis that included above- and belowground plant biomass, drainage, $NH_4^+$, $NO_3^-$, net N-mineralization, and potential extracellular enzyme activity was used to identify the consistency of the effects of soil sterilization and amendments on the samples. For the most part, field samples clustered separately and were distinct from the sterilized samples (Figure 4). Forty-six of 72 field samples fell into three main branches of the dendrogram produced from the cluster analysis. Only two sterilized samples were grouped in these branches. Additionally, many sterilized samples grouped exclusively together as observed in the center section of the dendrogram in Figure 4.

Many woodchip-amended samples clustered separately from the other amended treatments (Figure 5). Forty-one out of forty-eight woodchip samples clustered together on three main branches of the dendrogram. Some biochar and non-amended samples clustered with similar amendment treatments but this distribution is not as discrete as the woodchip-amended samples. Biochar and non-amended samples have a more heterogeneous distribution throughout the tree than the woodchip-amended samples.

Both field and sterilized soils amended with woodchips are more similar to other samples with the same treatment than they are to samples that received an alternate treatment (Figure 4 and Figure 5). Additionally, field samples amended with woodchips are more similar to sterilized samples amended with woodchips (and vice versa) than they are to the biochar or non-amended treatments.

The first two components of the PCA explained 60.18% of the variance in the data (proportion of the variance for PC1 and PC2 was 0.390 and 0.212 respectively). PC1 had the highest loadings with all the variables that measured extracellular enzyme activity, while PC2 had the highest (but mixed) loadings with above- and below-ground biomass, drainage, ammonium, nitrate, and net N-mineralization (Table 5). These two sets of variables separately account for a large percentage of variation in the dataset. When the samples were plotted on the first two components sterilized samples fell on the left side of PC1 and sterilized samples fell on the right side of PC1. Along PC2, woodchip samples clustered together in the center portion of the graph while biochar and non-amended samples did not have a discrete spatial pattern (Figure 6).

## 4. Discussion

When considering reestablishment plans, it is essential to understand the effects soil treatments will have on both plants and microbial communities. Plants and the soils they grow in are in constant contact with one another such that, changes in one will have effects on the other. Here we manipulated soils by sterilizing them and adding amendments to study how these changes affect both plants and soil microbial communities.

The woodchip amended soils resulted in the lowest plant biomass compared to all other soil treatments. These results are similar to other studies where woodchips reduced plant cover by about 7% compared to a control with no woodchips (Cotts et al., 1991; Miller and Seastedt, 2009; Petersen et al., 2004). By changing DOC and TN, woodchips may have caused inhospitable conditions by reducing soil water and nutrient availability. The juniper tree mulch used in this experiment has a high lignin

content in cell walls, which has been shown to be highly resistant to decomposition compared to alternate mulches (Duryea et al., 1999). This would lead to higher degree of recalcitrant carbon, which is not easily broken down by most soil microorganisms (Sollins et al., 1996). The increased concentration of recalcitrant carbon could also be an explanation for the decrease in in carbon mineralizing enzyme activities in the filed soils amended with woodchips (Table 3) (Ylla et al., 2012).

In the sterilized soils, our results indicate that there was also the highest drainage and lowest soil water content in the woodchip-amended soils. Initial soil water content was 75% lower in this treatment than the next lowest observation (e.g., sterile non-amended), and overall drainage was 22% higher. The poor water holding capacity of these soils may have been a main reason for the low plant establishment and biomass. It is possible that autoclaving soils may have caused the woodchips to adopt hydrophobic properties, contributing to the high drainage rates and low soil water content (Table 2). If hydrophobic properties did result from the autoclave sterilization this would limit water recharge in the soils, which would ultimately limit water availability to plants (Chalker-Scott, 2007). Additionally, sterilization of the woodchips could have aided in the decomposition of recalcitrant carbon. This effect is seen in the increase of XYL, AG, and BG potential extracellular enzyme activities (Table 3) (Waldrop et al., 2013).

We expected the high C:N ratio of the woodchips to cause nitrogen immobilization, and ultimately limit nitrogen availability to plants (Rensburg and Morgenthal, 2004). On the contrary, the woodchip amended soils in this study have high N-mineralization rates. Miller and Seastedt found similar results in their study where inorganic nitrogen values increased 33% in woodchip amended plots relative to non-amended pots (Miller and Seastedt, 2009). Overall, however, the non-amended soils had the highest N-mineralization rates 2.97 ± 0.17 (Figure 3). N-mineralization rates of the biochar amended soils were 0.73 ± 0.27. A possible mechanism for this decrease in N-mineralization could be sorption of

nitrogen rich compounds into the biochar (Dempster et al., 2012). Again, high biochar C:N ratios could lead to nitrogen immobilization ultimately leading to a deficiency (Atkinson et al., 2010).

The biochar-amended soil resulted in the highest plant biomass for all plant functional types except the nitrogen-fixing shrub *C. eriophylla,* which had the highest biomass in the non-amended soils. The addition of biochar decreased water drainage rates, thereby improving soil water permeability (Asai et al., 2009; Atkinson et al., 2010). Additionally, biochars have been shown to both absorb nutrients in the soil and act as a habitat for microbes (Kookana et al., 2011; Pietikäinen et al., 2000). Biochars produced under different temperatures have been shown to have variable absorptive properties related to an increase in overall surface area (James et al., 2005). In the present study, it is possible that autoclaving the soils may have increased the overall surface areas of the biochar. This would lead to a greater degree of nutrient and water absorption resulting in higher plant growth (Table 1).

In the sterilized biochar and nonamended soils there was more belowground biomass than in the field counterparts. The autoclave sterilization in this study decreased bacterial cell counts by 29% relative to non-sterilized field soils. The removal of microorganisms through sterilization has been shown to simulate the growth of plants through nutrient pulses and by removing root parasites and pathogens (Cerligione et al., 1988). Additively, the absence of beneficial microbes such as mycorrhizae that aid plants in nutrient acquisition could simulate plant root growth. Fewer symbiotic root associates increases plant investment into root growth in order to forage and acquire nutrients (Clarkson, 1985; Dakora and Phillips, 2002).

All potential extracellular enzyme activity was higher in the biochar amended soils relative to non-amended field soils. This result is consistent with previous studies that examined the effect of biochar addition to soils on potential extracellular enzyme activities and found an increase in activity with the addition of biochar (Awad et al., 2012; Bailey et al., 2011). Zimmerman et al., 2011 found that biochar additions cause an increase in the rates of carbon mineralization. All enzymes involved in the

degradation of carbon measured in this experiment had at least a 40% increase in activity when biochar was added relative to the non-amended filed soils (Table 3).

Although evidence from the literature suggests plant functional groups should have an effect on soil enzyme activities, our results did not support this. It is possible that the duration of this experiment may not have been long enough to foster particular associations between plants and soil microbes. Many studies showing plant acquisition of microbial associates expand durations longer than the time period researched in this experiment (Bakker et al., 2012; Bever et al., 2012; Schnitzer et al., 2011). Soil treatments (sterilization and amendments) have a greater impact on enzyme activities than plant species or plant biomass (Figures 4 and 5). Cluster analysis of the measured variables showed that sterilization of soils and the addition of amendments drove trends in the data such that, samples that clustered together were more often than not from the same soil treatment. This trend was especially evident in the woodchip-amended soils. The effects of biochar and non-amended soils seem to be more heterogeneous.

The results of the PCA further illustrate that plant biomass and soil microbial activity were not tightly coupled. The loadings for PC1 were highest in all measured potential extracellular enzyme activities, while the loadings for PC2 were highest in all other measured variables. This implies that each of these sets of variable separately account for the total overall variation in the dataset.

With current trends in land use and land cover change, it is important to develop well informed restoration plants in order to maintain plant diversity and overall cover. These plans for degraded landscapes can be improved upon by considering plant-soil interactions, the affect amendments may have, and the overall status of the soil. In this system, the soil treatments had more of an effect on plant growth and establishment than the soil microbial communities. We found that soil sterilization resulted in a decrease of microbial activity while the addition of amendments caused changes in activity that can be explained by changes in nutrient availability, but more research is needed to fully resolve this

relationship. Amendments and sterilization both had effects on soil moisture and water drainage that are reflected in plant growth patterns. Woodchip amended soils had the lowest overall plant growth. Biochar amended soils had highest plant growth when soils were sterilized, although this was not significant from other treatments. More research is needed to fully resolve the effects soil sterilization and amendments have on plant growth and microbial community activity. Preliminary results indicate that when soil quality is reduced the addition of amendments can stimulate microbial community activity and plant growth under certain circumstances.

**Tables**

Table 1. Focal plant species. The plants in the community pots (codes C1-C4) are delineated by the codes for each individual species. WSPG- Warm season perennial grass, CSPG- Cool season perennial grass, AF- annual forb, PF- perennial forb, and SH-shrub

| Scientific Name | Family | Code | C-cycle | Group | Common Name |
|---|---|---|---|---|---|
| *Eragrostis intermedia* | Poaceae | ERIN | C4 | WSPG | Plains lovegrass |
| *Bouteloua gracilis* | Poaceae | BOGR | C4 | WSPG | Blue grama grass |
| *Hilaria belangeri* | Poaceae | HIBE | C4 | WSPG | Curly-mesquite |
| *Digitaria californica* | Poaceae | DICA | C4 | WSPG | Arizona cottontop |
| *Bouteloua curtipendula* | Poaceae | BOCU | C4 | WSPG | Sideoats Grama |
| *Leptochloa dubia* | Poaceae | LEDU | C4 | WSPG | Green sprangletop |
| *Elymus elymoides* | Poaceae | ELEL | C3 | CSPG | Squirreltail |
| *Eschscholzia mexicana* | Papaveraceae | ESME | C3 | AF | Mexican poppy |
| *Baileya multiradiata* | Asteraceae | BAMU | C3 | PF | Desert marigold |
| *Calliandra eriophylla* | Fabaceae | CAER | C3 | SH | Fairy duster |
| BOCU, DICA, LEDU | | COM1 | | | |
| HIBE, BAMU, CAER | | COM2 | | | |
| ELEL, ESME, BAMU | | COM3 | | | |
| BOCU, ELEL, ESME | | COM4 | | | |

Table 2. Initial soil measurements. qPCR values are recorded as total number of bacterial cells/g dry soil. Organic matter percentage (OM%) by loss on ignition is measured as the difference in weight before and after ignition at 400°C divided by the before weight x 100. Soil Water Content (SWC) is recorded in g. It was measured as the difference in weight before and after incubation for 24h at 105°C. pH is presented on a logarithmic scale. Microbial biomass C and N are determined as the difference in values before and after 48h incubation with ethanol free chloroform and are presented as mg/kg. Finally, drainage is presented in g and is determined as the difference in weights immediately after watering and 30 minutes later. Weight immediately after watering is calculated as weight of pot before watering+8g (representing 8mL DI water). Dissolved organic carbon (DOC) and total nitrogen (TN) are determined as the concentration of carbon or nitrogen before incubation with ethanol free chloroform and are presented as mg/kg.

| Amendment | Sterilization | qPCR | OM% | SWC | pH | microbial biomass C | microbial biomass N | drainage | DOC | TN |
|---|---|---|---|---|---|---|---|---|---|---|
| Nonamended | Field | 2.98E+07 | 3.86 | 2.7 | 8.335 | 1.08 | 0.27 | 2.63 | 1.43 | 0.10 |
| | Sterile | 2.12E+07 | 4.15 | 1.6 | 8.265 | 0.47 | 0.33 | 2.99 | 3.33 | 0.87 |
| Biochar (4%) | Field | 6.24E+07 | 6.29 | 2.5 | 8.17 | 1.12 | 0.23 | 2.32 | 1.96 | 0.16 |
| | Sterile | 1.54E+07 | 3.89 | 1.8 | 8.3 | 0.13 | 0.30 | 1.75 | 4.25 | 1.00 |
| Woodchips (8%) | Field | 6.17E+07 | 7.24 | 2.4 | 7.985 | 5.34 | 0.40 | 2.62 | 2.48 | 0.03 |
| | Sterile | 1.35E+05 | 7.19 | 0.4 | 7.595 | 1.19 | 0.35 | 3.84 | 15.98 | 0.67 |

Table 3. Potential soil extracellular enzyme activity measured at 25°C. Values are means (nmol $h^{-1}$ g SOM) ± standard error values. Letters represent TukeyHSD post hoc significance test within each substrate. C:N ratio was calculated as: sum of (CB+XYL+AG+BG) activity / sum of (NAG+LAP) activity; C:P ratio was calculated as: sum of (CB+XYL+AG+BG) activity / PHOS activity; N:P ratio was calculated as: sum of (NAG+LAP)

| Amendment | Sterilization | CB | XYL | AG | BG | NAG | LAP | PHOS | C:N | C:P | N:P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nonamended | Field | 1.28 ± 0.18b | 3.46 ± 0.39b | 1.28 ± 0.16ab | 9.99 ± 0.84b | 1.30 ± 0.16a | 38.33 ± 2.17b | 50.51 ± 4.64b | 0.42 ± 0.04ab | 0.36 ± 0.05a | 1.01 ± 0.14a |
| | Sterile | 0.41 ± 0.07c | 0.44 ± 0.07c | 0.69 ± 0.10bc | 1.15 ± 0.19c | 0.62 ± 0.11b | 10.68 ± 0.64d | 22.82 ± 2.47c | 0.25 ± 0.04ab | 0.10 ± 0.01c | 0.71 ± 0.09ab |
| Biochar (4%) | Field | 2.18 ± 0.39a | 5.60 ± 0.93a | 1.80 ± 0.29a | 18.68 ± 1.85a | 1.87 ± 0.24a | 63.58 ± 3.92a | 92.41 ± 8.47a | 0.42 ± 0.03ab | 0.30 ± 0.02ab | 0.79 ± 0.05ab |
| | Sterile | 0.25 ± 0.06c | 0.68 ± 0.14c | 0.51 ± 0.10c | 1.19 ± 0.16c | 0.46 ± 0.11b | 14.00 ± 1.59d | 30.39 ± 2.75bc | 0.20 ± 0.04b | 0.08 ± 0.01c | 0.49 ± 0.04b |
| Woodchips (8%) | Field | 0.95 ± 0.13bc | 3.26 ± 0.29b | 1.02 ± 0.11bc | 9.76 ± 0.55b | 1.72 ± 0.20a | 64.02 ± 2.77a | 75.48 ± 5.73a | 0.23 ± 0.02b | 0.21 ± 0.01bc | 1.00 ± 0.08a |
| | Sterile | 0.30 ± 0.07c | 0.97 ± 0.16c | 0.82 ± 0.15bc | 9.35 ± 2.64b | 0.58 ± 0.10b | 24.08 ± 1.09c | 30.92 ± 3.31bc | 0.47 ± 0.11a | 0.38 ± 0.07a | 1.09 ± 0.13a |

activity/ PHOS activity;

Table 4. ANOVA results of potential soil extracellular enzyme activity. Results are listed as F values with df and p-values for ANOVA.

| | Treatment | Amendment | Treatment x Amendment |
|---|---|---|---|
| CB | $F_{1,192} = 54.795$; $p < 0.0001$ | $F_{2,192} = 4.868$; $p < 0.01$ | $F_{2,192} = 6.47$; $p < 0.01$ |
| XYL | $F_{1,192} = 91.047$; $p < 0.0001$ | $F_{2,192} = 4.345$; $p = 0.014$ | $F_{2,192} = 4.776$; $p < 0.01$ |
| AG | $F_{1,192} = 26.821$; $p < 0.0001$ | $F_{2,192} = 1.121$; $p = 0.328$ | $F_{2,192} = 5.611$; $p < 0.01$ |
| BG | $F_{1,192} = 62.354$; $p < 0.0001$ | $F_{2,192} = 6.106$; $p < 0.01$ | $F_{2,192} = 19.073$; $p < 0.0001$ |
| NAG | $F_{1,192} = 65.496$; $p < 0.0001$ | $F_{2,192} = 1.027$; $p = 0.360$ | $F_{2,192} = 2.539$; $p = 0.082$ |
| LAP | $F_{1,192} = 430.67$; $p < 0.0001$ | $F_{2,192} = 38.48$; $p < 0.0001$ | $F_{2,192} = 11.36$; $p < 0.0001$ |
| PHOS | $F_{1,192} = 119.66$ $p < 0.0001$ | $F_{2,192} = 12.64$; $p < 0.0001$ | $F_{2,192} = 5.87$; $p < 0.01$ |
| C:N | $F_{1,192} = 1.229$; $p = 0.269$ | $F_{2,192} = 0.295$; $p = 0.745$ | $F_{2,192} = 10.191$; $p < 0.0001$ |
| C:P | $F_{1,192} = 13.267$; $p < 0.001$ | $F_{2,192} = 4.414$; $p = 0.013$ | $F_{2,192} = 22.381$; $p < 0.0001$ |
| N:P | $F_{1,192} = 4.777$; $p = 0.030$ | $F_{2,192} = 8.979$; $p < 0.001$ | $F_{2,192} = 2.942$; $p = 0.055$ |

Table 5. PCA loadings for the first three components resulting from the PCA.

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| aboveground | -0.055 | 0.502 | -0.264 |
| belowground | -0.003 | 0.488 | -0.302 |
| drainage | -0.007 | 0.324 | -0.260 |
| CB | -0.394 | 0.055 | -0.106 |
| NAG | -0.345 | -0.063 | 0.082 |
| XYL | -0.406 | 0.043 | 0.022 |
| AG | -0.375 | 0.037 | -0.055 |
| BG | -0.371 | -0.001 | 0.049 |
| LAP | -0.349 | -0.021 | 0.116 |
| PHOS | -0.384 | -0.014 | -0.019 |
| $NH_4^+$ | 0.097 | 0.320 | -0.206 |
| $NO_3^-$ | -0.019 | -0.349 | -0.622 |
| N-mineralization | -0.030 | -0.412 | -0.552 |

**Figures**

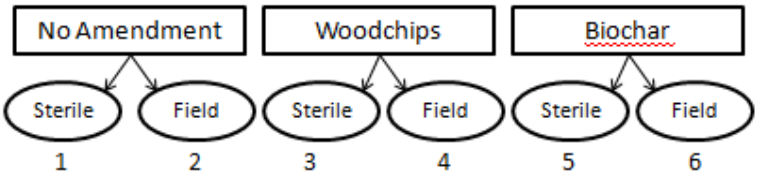


Figure 1: Six soil treatments used in the study included amending field-collected soil with biochar, woodchips, or no addition and autoclave-sterilizing half of the treatments to reduce the live soil microbial community.

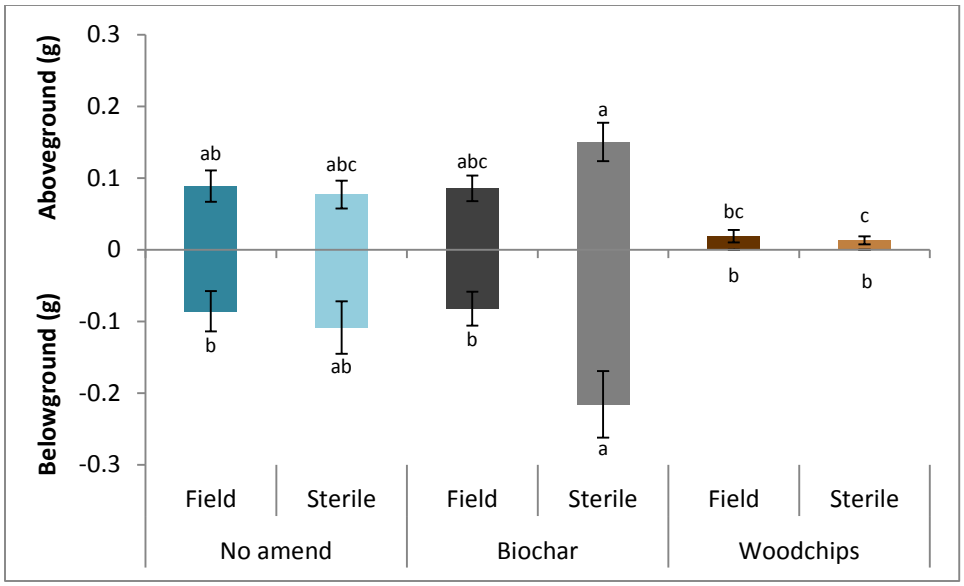


Figure 2: Aboveground and belowground plant biomass in the six soil treatments. Bars represent average biomass (g). Error bars indicate standard error (SE) values of the mean. Letters indicate TukeyHSD post hoc significance test results within aboveground biomass and belowground biomass.

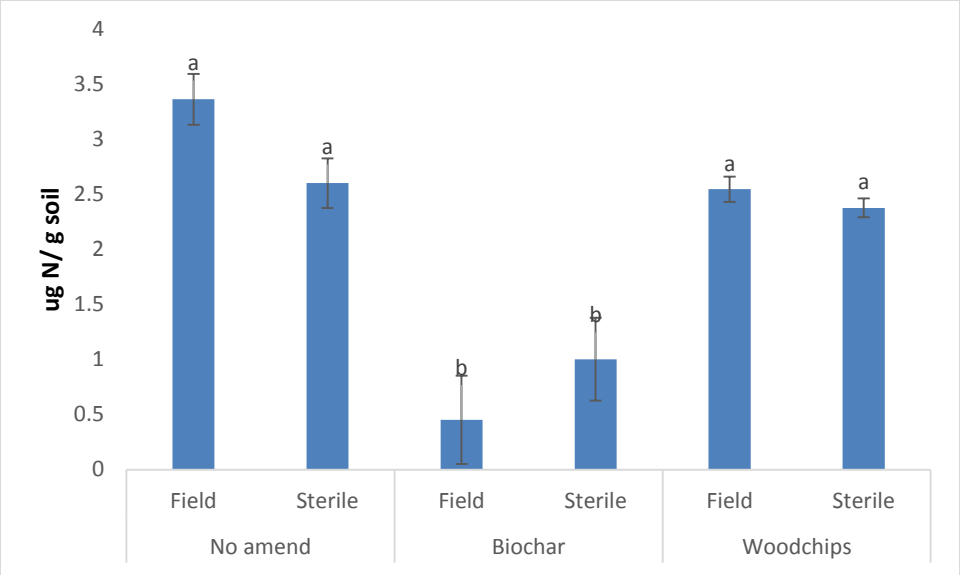


Figure 3. Net N-mineralization calculated by: ($NH_4^+$+$NO_3^-$) post-incubation - ($NH_4^+$+$NO_3^-$) pre-incubation; Bars represent average net N mineralization (ug N/gsoil/week). Error bars indicate standard error (SE) values of the mean. Letters indicate TukeyHSD post hoc test.

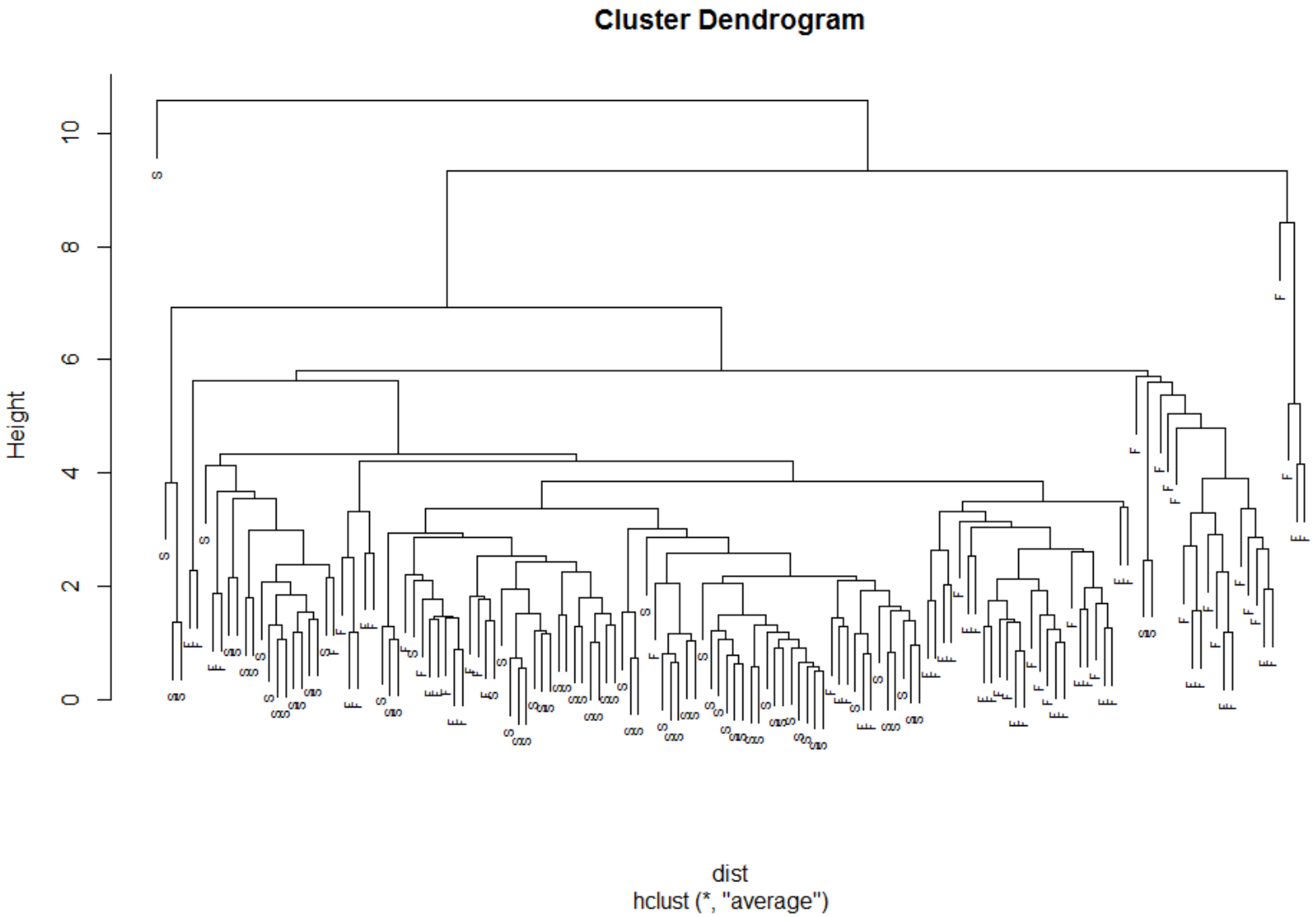


Figure 4. UPGMA dendrogram produced from Euclidean distance matrix of measured variables. Labeled according to sterilization treatment. F- unsterilized field soils and S- sterilized soil.

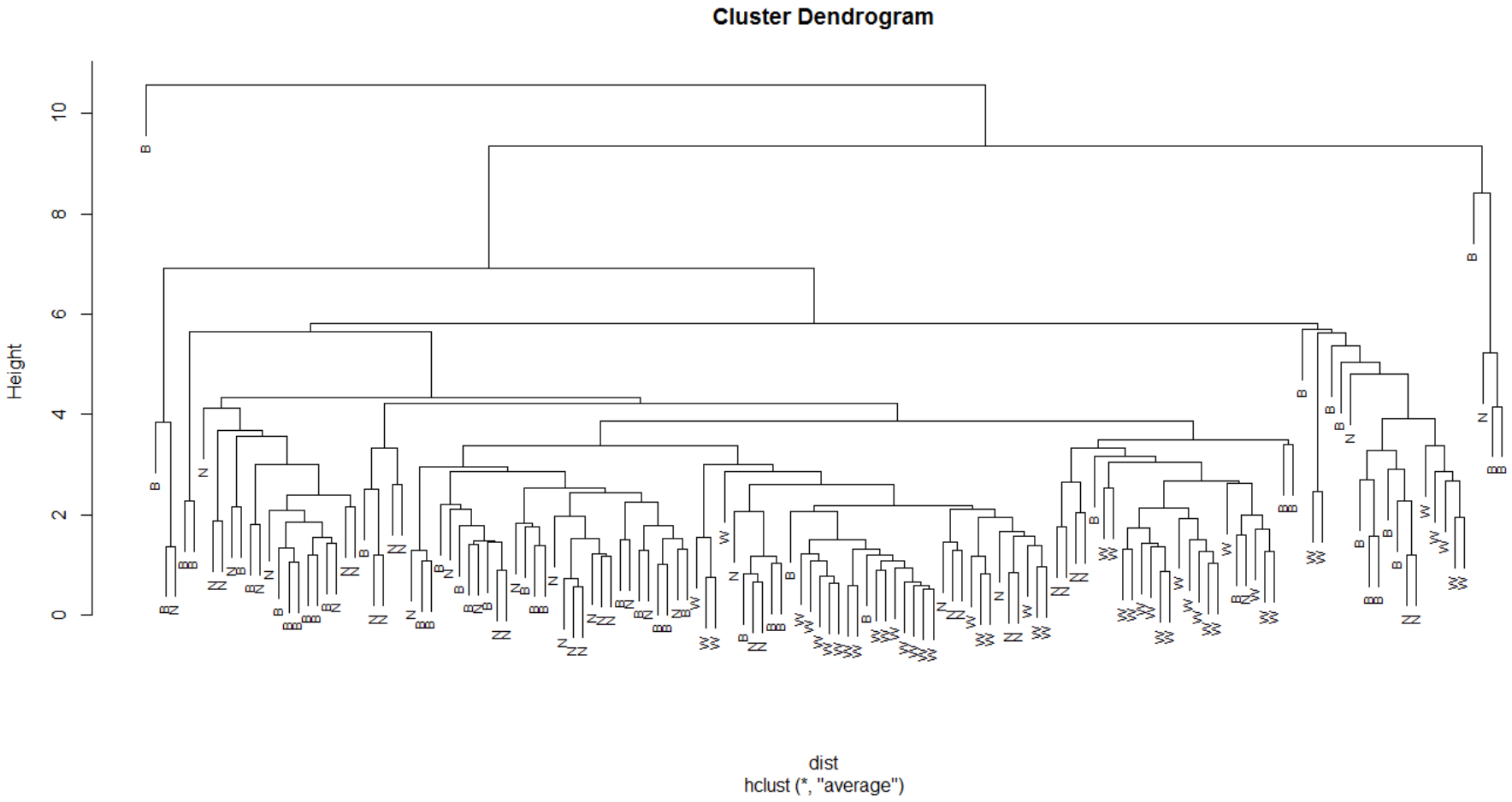


Figure 5. UPGMA dendrogram produced from Euclidean distance matrix of measured variables. Labeled according to amendment treatment. N- non-amended soil, B- biochar amended soil, and W- woodchip amended soil.

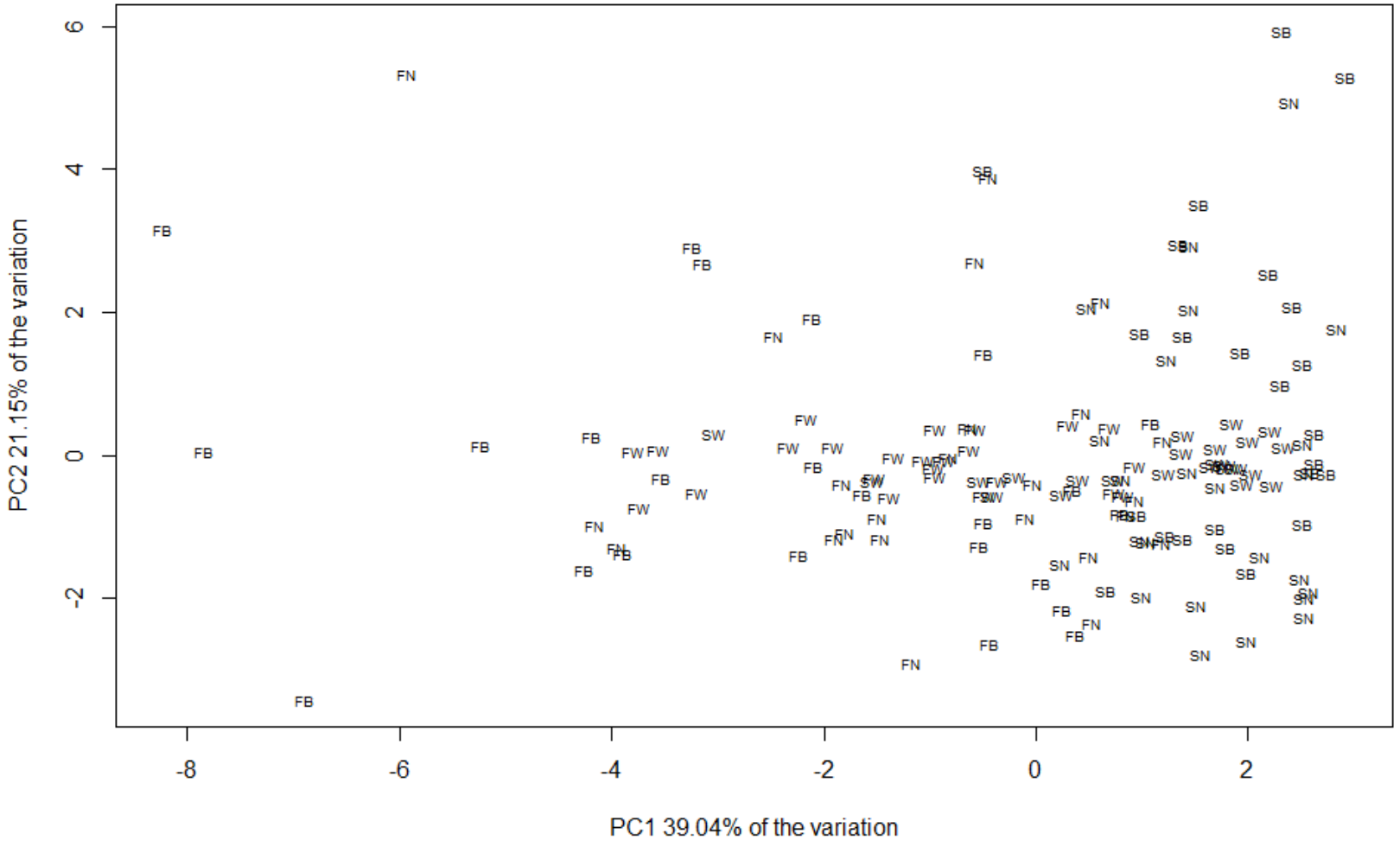


Figure 6. Scatterplot matrix of the first two components from the PCA. Points are symbolized according to soil treatments. F- filed soils, S- sterilized soils; N-non-amended, B- biochar amended, and W- woodchip amended.

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