**Woodchip and biochar amendments differentially influence microbial responses, but do not enhance plant recovery in disturbed semiarid soils**

Noelle J. Espinosa¹,², David J. P. Moore¹, Craig Rasmussen³, Jeffrey S. Fehmi¹, Rachel E. Gallery¹,⁴

Restoration presents a global challenge in drylands (arid and semiarid ecosystems) where uses can range from exclusive conservation to open-pit mining and restoration practices are constrained by scarce, unpredictable precipitation, and high ambient temperatures. Adding woodchip amendments to soils is a common strategy for mitigating soil degradation as amendments may enhance soil carbon and increase plant cover. We assessed the effect of surface or incorporated woodchip addition and incorporated wood-derived biochar on soil carbon dynamics and microbial activities as well as plant cover in semiarid soils that had been removed and replaced. We found that woodchips at the soil surface increased soil organic carbon (SOC), and both surface and incorporated woodchips increased the dissolved organic carbon (DOC) content. The incorporation of woodchips inhibited plant cover yet increased soil CO₂ efflux and dissolved organic matter stoichiometry. Surface woodchips also significantly enhanced microbial activities but not plant cover. A significant amount of the soil efflux in response to incorporating woodchips was explained by plant cover and exoenzyme activities, but this was not the case for other amendment treatments. Biochar, thought to be more resistant to decomposition, neither stimulated nor reduced microbial activities or plant cover and did not influence SOC or DOC. Our findings demonstrate that the influence of woodchip amendments on microbial processes and soil carbon dynamics depends on the location of application and that coarse fast-pyrolysis biochar has limited influence on soil processes over a 22-month study in a water-limited ecosystem.

**Key words:** drylands, exoenzyme and extracellular enzyme activities, revegetation, soil management, soil respiration, Sonoran Desert

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**Implications for Practice**

- A thin surface application of woodchips is recommended because this treatment supported establishment of plant cover as well as increased soil organic and dissolved carbon which may lead to soil carbon storage.
- Incorporating biochar into the soil did not greatly benefit reestablishment of plant cover in degraded semiarid soils although it did store carbon in the sense that obdurate carbon got buried on the site.
- Incorporating woodchips into degraded soils is not recommended because it suppressed plant cover.

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**Introduction**

Restoration of degraded soils in drylands (arid and semiarid ecosystems) is challenged by scarce, unpredictable precipitation and high ambient temperatures (Reynolds et al. 2007). Organic amendments such as coarse woodchips or biochar have been used to aid in reestablishing vegetative cover and rooting systems by improving soil moisture dynamics (Benigno et al. 2013; Breton et al. 2016) or nutrient-holding capacity in degraded soils (Glaser et al. 2001). Amendments may also sequester soil carbon (C) by benefiting soil biological activities that influence soil C stocks and fluxes (Masciandaro et al. 2004; Biederman & Whisenant 2011; López et al. 2014; Scotti et al. 2015); outcomes that have historically been a minor focus of restoration efforts. Quantifying the influence of amendments on soil quality indicators such as organic carbon and soil nutrients, in combination with microbial activities, may identify strategies that enhance the potential for revegetation, soil reconstruction, and soil C storage (An et al. 2013; Muñoz-Rojas et al. 2016).

Amending soils with woodchips or biochar can be cost effective and feasible to implement on a large scale. Wood waste...
from thinning and invasive plant management can produce excess materials (Tahboub et al. 2008; Biederman & Whisenant 2011) that are an ideal and local source of woodchips. While excess wood is often burned as an economic means of disposal, instead, an incomplete pyrolysis produces biochar that can have benefits to plant growth and soil physical and biological properties (Shrestha et al. 2010). Thanks to a growing body of research demonstrating the potential for woodchips and biochar amendments to improve soil physical properties and revegetation success (Sanborn et al. 2004; Lehmann et al. 2006), using these materials for dryland restoration may have an added benefit as a much-needed avenue for managing these waste streams.

The type and placement of amendments, at the soil surface or incorporated into soil, can differentially influence soil properties and soil carbon and nitrogen (N) cycling (Biederman & Whisenant 2011; Prober et al. 2014). For example, amendments applied to the soil surface have been used to reduce soil erosion, increase plant recruitment, and increase soil nutrient availability (Chalker-Scott 2007; Biederman & Harpole 2013). Alternatively, incorporating woodchips into the soil has been shown to increase soil moisture and plant growth rates (Benigno et al. 2013); however, it has also been shown to enhance soil drainage which can potentially result in low plant-available water or N (Gebhardt et al. 2017). Meanwhile, biochar applications have been shown to increase soil C and reduce nutrient leaching (Biederman & Harpole 2013). The chemical nature of biochar is resistant to microbial degradation, with greater adsorption and slower release of nutrients resulting in longer-term effects compared to unpyrolyzed wood (Lehmann & Joseph 2015). However, these attributes are highly dependent on production conditions (pyrolysis temperatures, particle size, source material; Zimmerman 2010; Wang et al. 2016), adding to the necessity of continued experimentation using a variety of biochar types. Monitoring changes in vegetation as well as soil biogeochemical indicators (e.g. soil C, soil N, soil organic content) in response to different types and locations of amendments is useful in assessing their direct influence on soils (Mayor et al. 2013). However, these indicators interact with and are mediated by microbes (Jastrow et al. 2006; Lange et al. 2015; Mbuyita et al. 2015). Therefore, understanding changes in microbial metabolism, biomass, and function in response to different types and locations of amendments may provide additional insight into the long-term efficacy of these soil management practices (Muñoz-Rojas et al. 2016).

While woodchip and biochar amendments physically add C macromolecules to the soil subsurface (e.g. lignin, cellulose, and other polysaccharides), these amendments may have the additional benefit of potentially stabilizing soil C via microbial metabolic processes and soil mineral interactions (conceptually referred to as the microbial carbon pump; Liang et al. 2017). Kallenbach et al. (2016) demonstrated that organic molecules of microbial origin resulting from the decomposition of dissolved organic matter (DOM) can be relatively more stable compared to untransformed plant-derived molecules. These microbial molecules can thus significantly contribute to C stabilization in mineral soils (Kalbitz & Kaiser 2008; Liang et al. 2017). Consequently, the physical addition of plant-derived C in the form of charred and uncharred wood amendments may provide a slow release feedstock to the microbial carbon pump, which may sustain production of microbial-derived soil C. Research in temperate ecosystems has shown that DOM-derived C can represent up to 50% of total soil C (Kalbitz & Kaiser 2008), yet studies that examine these dynamics in water-limited ecosystems are rarer. Although the large soil-volume ratio of the woodchips and biochar may be an impediment to decomposition (Biederman & Whisenant 2011), incorporating woodchips into the surface soil increases surface area in contact with the decomposer community, which can stimulate dissolved organic carbon (DOC) production (Kalbitz & Kaiser 2008) and biogeochemical activity (Hewins et al. 2017).

The objective of this study was to assess how woodchip and biochar amendments influence soil C dynamics, microbial activities, and revegetation with a focus on semiarid soils degraded through the surface being removed and replaced. We hypothesized that surface-applied woodchips would enhance plant cover by improving soil moisture but not influence soil biogeochemistry. We hypothesized that incorporating amendments would change physical soil structure creating soil microenvironments that enable microbial activities, biomass growth, and enhance nutrient cycling, thus improving plant cover compared to non-amended soils. We expected that biochar would initially dampen microbial activities due to its high reactive surface area and ability to adsorb soil nutrients, and that beneficial effects on soil carbon may not be resolved in the short duration of this analysis. Given that soil efflux is the sum of plant root respiration and heterotrophic microbial respiration, we expect that the relationships of these three variables will represent plant–microbe interactions in response to soil amendments. We expected that responses to treatments would likely only be observable following monsoon events since water limitation periodically inhibits microbial activities in semiarid climates; therefore, we sampled soils following the two monsoon seasons in the Sonoran Desert.

**Methods**

**Research Site**

The study was conducted in Tucson, Arizona, at the Campus Agricultural Center of the University of Arizona (32°16'51.63"N, 110°56'11.34"W, at an elevation of 713 m above sea level). The long-term mean, minimum, and maximum annual temperatures are 20.7, 11.8, and 29.7°C and mean annual precipitation is 305 mm (Arizona Meteorological Network 2013). Field plots were established in June of 2013.

**Experimental Design**

This study is part of a long-term experiment designed to test the potential of woody amendments to aid in the restoration of vegetation and biogeochemical cycling in heavily disturbed soils. The soils used in this study are common to an area intended for proposed copper mining activities, which will excavate soils on a large scale, destroying soil horizons and structure, and stockpile soil for future use in restoration. The two soils used
in this study are classified as an Ustic Haplorgid and Aridic Calcisoll (Hathaway and Chiricahua soil series, respectively; Soil Survey Staff, 2014). To mimic this potential disturbance, soils were excavated mechanically using a small front-end loader to a depth of approximately 1.5 m below the surface. Soils were mixed separately to homogenize soil horizons and remove soil structure and transported to the Campus Agricultural Center where replicate field plots were established in June 2013. Both soils were sandy loam in texture (Hathaway soil series consisted of 59.7% sand, 30.9% silt, and 9.4% clay, while the Chiricahua series consisted of 69.1% sand, 24.4% silt, and 6.4% clay); however, they differed in rock fragment content, pH, and KCl extractable NH₄ and NO₃ (Table S1; Rasmussen et al. 2015). Soils were filled into excavated field plots (approximately 1 x 1 m and 30 cm depth) lined with porous geotextile fabric, which enabled water to drain but inhibited the mixing of the experimental soil with the surrounding native soil.

Each of the two soil types received one of three treatments: woodchips added to the plot surface (surface), woodchips incorporated into the soil, or biochar incorporated into the soil. The woodchips and biochar were incorporated into soils using a cement mixer prior to placing the homogenized soil-amendment mix into the field plots. Control plots were established as described above but without addition of an amendment. Each treatment was replicated four times for each soil type resulting in a total of 32 plots. Woodchips from Juniperus monosperma (Cupressaceae) were created from trees growing on the site where soil was collected. The source material from the site of disturbance replicates a real future scenario where the destruction of the area would create an excess of usable woody material. Green trees were chipped and applied to plots in June 2013 with a Vermeer BC600XL 6-in. Brush chipper (Pella, IA, U.S.A.). The particle sizes of the chips varied from coarse sawdust to fragments about 7.5 x 1.5 cm, with particles predominantly less than 1 cm in size. A detailed description of woodchip particle size is described by Fehmi et al. (this issue). Woodchips (C: N = 138:1, Table S2) were added to the surface treatment to cover approximately 80% of the soil surface. The ratio of incorporated woodchips or biochar to soil was 4% by weight. At the time of application, the uncharred woodchips were approximately 10.5% water by weight compared to woodchips air dried at ambient temperature for 6 months (see Table S3). Biochar used in the experiment (C = 69.2%; aromaticity index = 211.64, Table S2) was produced from fast-pyrolysis of a mixture of hardwood tree species from the northeastern United States at ambient temperature for 6 months (see Table S3). Biochar and woodchips were applied to the surface plots and mixed separately to homogenize soil horizons and remove soil structure. Biochar and woodchips were incorporated in the soil, or biochar incorporated into the soil. The experimental soil with the surrounding native soil. The soil was forced to white and vegetation to black. The ratio of

Soil and Vegetation Sampling and Processing

The Sonoran Desert receives the largest proportion of its precipitation during the summer monsoons (Crimmins 2006). Soils for biogeochemical and microbial analyses were collected from all 32 plots (n = 4 per treatment) in November 2014 following the end of summer monsoons (approximately June–September; Fig. S1) and in April 2015 following the end of the winter rainy season (Fig. S1; approximately November–March). Soils were collected from the top 5 cm of each plot and stored on ice until they were transported to the laboratory, sieved at 2 mm to remove rock, woodchip, and biochar fragments, and stored at 20°C until analysis of soil properties and microbial activities (Hewins et al. 2016). Subsamples from each plot were used to determine pH, gravimetric water content (GWC), and soil carbon content. Soil pH was measured with 5 g of soil mixed with 10 mL deionized water (sympHony Model SB20, VWR International, Radnor, PA, U.S.A.; Hendershot et al. 1993). Soils were shaken for 15 minutes and allowed to settle for 30 minutes before the measurement was taken. Percent moisture content was determined by weighing 5 g of field moist soils prior to drying for 48 hours at 70°C. Soil organic matter (SOM) was quantified as loss on ignition (LOI) using 5 g oven-dried soils that were transferred to a furnace for combustion for 5 hours at 500°C (Heiri et al. 2001). Soil organic carbon (SOC) was determined from LOI SOM using the conversion equation (SOC = a_T × (LOI – b_T × C) proposed by Hoogsteen et al. (2015), which accounts for ignition temperature and structural water loss from clays. Briefly, a_T is an SOM to SOC conversion factor for a given ignition temperature, LOI is the mass loss of SOM (%), b_T is a clay correction factor for a given ignition temperature, and C is the clay content of the soil. The LOI method was used to determine relative differences between treatments and we recognize this method may not accurately determined absolute SOM based on ignition temperature and duration (Hoogsteen et al. 2015).

Percent plant cover or percentage of soil covered by any part of the plant was determined by image analysis. Images were converted to black and white (binary values 0 and 255) where soil was forced to white and vegetation to black. The ratio of
Microbial Biomass, Organic C, and Exoenzymes

Microbial biomass, DOC, and total dissolved N (TDN) were determined using a modified chloroform-fumigation extraction method (Vance et al. 1987), with deionized H2O as the extractant (Haney et al. 2001). In addition to sieving soils to 2 mm, plant seeds, roots, and wood fragments smaller than 2 mm were removed with tweezers from two subsamples of 5 g each. One subsample was fumigated with 2 mL of chloroform and incubated at room temperature for 24 hours. Both subsamples then received 25 mL dH2O before agitation on a shaker at 200 rpm for 1 hour. Extracts were filtered through carbon-free glass fiber filters (Whatman GF/C). Extracts were analyzed using a Shimadzu TOC-5000A equipped with a total dissolved nitrogen module (Shimadzu Scientific Instruments, Inc., Columbia, MD, U.S.A.). The term DOC is commonly used to describe two distinct forms of dissolved organics: (1) the total water extractable organic carbon (WEOC) produced from laboratory extractions, or (2) the extant DOC existing in the liquid phase of a soil located in in situ macro and micropores also referred to as DOC (Li et al. 2018). This overlap in usage of the term can create confusion and inhibit comparison of WEOC dynamics across studies. In the present study we refer to laboratory-extracted dissolved material as DOC or TDN for carbon or nitrogen, respectively.

Hydrolytic exoenzyme assays were performed for all plots and all collection dates (Table S4). Soils were analyzed for the total potential activities of seven hydrolytic exoenzymes using the 96-well plate fluorescence technique (Saiya-Cork et al. 2002). Soils were grouped pH and assayed with pH buffers according to their respective in situ soil pH. In parallel with sample analysis, linear standard curves were created with known substrate concentrations and were used to determine potential exoenzyme activities as nM g-dry soil⁻¹ hour⁻¹. A geometric mean enzyme activity (GMEA) was calculated using all seven activities for each sample following Chen et al. (2019). The GMEA integrates the seven exoenzyme activity variables into a single summary variable for soil exoenzyme activities.

Soil CO2 Efflux

Soil efflux, a proxy for the respiration of autotrophic and heterotrophic organisms in soil, is defined as the efflux of CO2 from a known area of soil per unit time. Soil efflux was measured in the field during each sampling event using an infrared gas analyzer (IRGA) with a 9.55 cm soil CO2 flux chamber (LI-6400-09, LiCor, Inc., Lincoln, NE, U.S.A.). To ensure that all soil efflux measurements were made under the same environmental conditions, we minimized the duration of sampling to 4 hours during midday by using three LI-6400 machines at each sampling event. To avoid instrument bias, we measured each treatment with each IRGA, rotating through treatments throughout each sampling day. Soil efflux was measured by connecting the soil chamber (LI-6400-09) to permanently installed polyvinyl chloride (PVC) collars which were installed at least 2 weeks prior to the first IRGA measurement and remained in place throughout the study. To avoid disturbing the soil near the collars, soil sampling occurred at least 20 cm from any PVC collar. Any vegetation growing within the collars were cut at the soil surface no less than 24 hours before soil efflux measurements were taken. Efflux measurements were made by reducing CO2 in the chamber to between 5 and 10 ppm below ambient CO2 conditions and recording the rate of CO2 build-up in the chamber to 5–10 ppm above ambient CO2 using the IRGA. For each plot this was replicated three times and averaged for each plot.

Statistics

Data were transformed to approximate normality using a natural log transformation with the exception of plant cover, which was square-root transformed, and DOC, TDN, and P-EE, which were transformed by λ values estimated using Tukey’s ladder of powers (λ = −0.2, 0.075, & 0.175, respectively). Untransformed data and means were used in figures and reported in the text to make interpretations straightforward. Multivariate analysis of variance (MANOVA) was used to determine differences among amendment treatments, soil types, and sample dates for plant cover, soil efflux, MBC, C, N, and P exoenzymes, SOC, DOC, TDN, and DOC:TDN (Table S5 and S6). Univariate analysis of variance (ANOVA) was conducted based on significant MANOVA results to determine differences between amendment treatments and the control. Post hoc Tukey’s honest significant differences tests were run to report pairwise comparisons. A single outlier was removed from MBC (>2,000 mg/g).

Principal component analyses (PCA) was performed on the response variables (GWC, soil pH, Plant Cover, Soil Efflux, SOC, DOC, C-EE, N-EE, MBC, DOC:TDN). PCA was used to determine the differences between amendment treatments and relationships among the observed variables across both soil types and sample dates. Only complete observations were used to calculate the PCA. Values were centered and scaled within the PCA. The PCA loadings were used to generate the biplot. See Supplemental Table S8 for summary results.

Correlation analyses were carried out with Pearson’s correlation method for the full dataset as well as for soil efflux, total plant cover, and GMEA within individual amendment treatments across both soil types and sample dates. Significance of correlations was adjusted for multiple comparisons using the Benjamini and Hochberg false discovery rate method (Benjamini & Yekutieli 2001). Statistical analyses were performed in R: Language for Statistical Computing (version 3.5.1) and R Studio (version 1.0.143; R Core Team 2018).

Results

Seasonal Influences of Plant and Microbial Responses

The magnitude of the monsoon significantly influenced plant cover, soil efflux, and microbial exoenzyme activities as well as DOC and TDN, but not SOC (Figs. 1 & 2). Both plant cover and soil CO2 efflux were higher following the larger summer
monsoon season (November 2014; Fig. S1) compared to the relatively smaller winter rainy season (49.5 ± 2.5% vs. 11.1 ± 2.5% for plant cover in November 2014 vs. April 2015, respectively). DOC, TDN, and the DOC to TDN ratio (DOC:TDN) were higher following the winter rains in April 2015 (4.45 ± 0.65 vs. 23.5 ± 6.3% for DOC, 0.55 ± 0.07 vs. 1.0 ± 0.20% for TDN, and 11.42 ± 2.5 vs. 27.62 ± 6.9% for DOC:TDN, for November 2014 vs. April 2015, respectively). Carbon-EE (exoenzyme), N-EE, and P-EE activities were significantly higher following the wetter summer monsoon season compared to the winter rainy season (137.58 ± 16.12 vs. 44.70 ± 3.67 for C-EE, 124.21 ± 9.85 vs. 51.05 ± 4.45 for N-EE, and 83.43 ± 12.86 vs. 29.04 ± 3.61 for P-EE for November 2014 vs. April 2015, respectively, Tables S6 & S7).

Soil Type Influences N and P Exoenzyme Activities but Not Plant Cover or Soil C

The two different soil types (Table S2) did not significantly influence plant cover, soil efflux, SOC, DOC, or DOC:TDN (p = 0.31;...
Table S5). Soil type was a significant factor for N and P exoenzyme activities (Fig. 2C & 2D; Fig. S2). Nitrogen-EE and P-EE activities in Chiricahua soil were significantly higher than activities in Hathaway soil (151.54 ± 14.8 and 96.88 ± 9.07 for N-EE, and 125.77 ± 20.14, and 41.09 ± 6.25 for P-EE for Chiricahua and Hathaway soil, respectively).

Positive, Neutral, and Negative Effects of Amendments

Amendment type influenced plant cover, soil efflux, SOC, DOC, and DOC:TDN in November 2014. Incorporated woodchips reduced plant cover by 31% and significantly increased soil efflux by 246% compared to the unamended control (Fig. 1A & 1B; Table S5). Soil organic carbon was significantly greater when woodchips were applied to the soil surface compared to the unamended control (Fig. 1C; Table S5). DOC was significantly greater in the incorporated and surface woodchip treatment compared to unamended controls (Fig. 1D; Table S5). The amendment treatments did not influence TDN (Fig. 1E; Table S5); however, DOC:TDN was only significantly greater when woodchips were incorporated into the soil compared to the unamended control (Fig. 1F; Table S5). Amendment type did not significantly influence C and N exoenzyme activities compared to the unamended control (Fig. 2B & 2C; Table S6 & S7). While sample date was significant, significant treatment effects were not present in April 2015 (p = 0.7; Figs. 1 & S3). An amendment by date interaction was present, but not for differences between sample date paired by amendment type (Table S5 & S6).

The PCA reduced 13 variables to two factors that accounted for 52.5% of the variance (Fig. 3). The loadings for PCA 1 were highest for N-EE, GMEA, Plant Cover, C-EE, and P-EE (loading values 0.40, 0.40, 0.35, 0.34, and 0.34, respectively; Table S8), while the loadings for PCA 2 were highest for DOC, MBC, and DOC:TDN (loading values 0.53, 0.47, and 0.44, respectively; Table S8). There was no discrete separation among amendment treatments along PCA 1; however, PCA 2 demonstrated a separation between surface and incorporated woodchips and the biochar and control treatments (Fig. 3).

The Pearson’s linear correlation coefficient was calculated between all combinations of the variables (Table 1). Across all amendments, soil types, and sample dates, soil efflux was most positively correlated with C, N, and P exoenzyme activities as well as the geometric mean of all exoenzyme activities. Soil efflux was also significantly correlated with plant cover, soil pH, and GWC. Carbon, N, and P exoenzyme activities were significantly positively correlated to each other as well as plant cover and GWC. Carbon exoenzyme activities were also negatively correlated with soil pH. Nitrogen and P exoenzyme activities were negatively correlated with DOC. The geometric mean of all exoenzyme activities was positively correlated with plant cover and GWC, while positively correlated with DOC. The DOC:TDN ratio was negatively correlated with plant cover and GWC, while positively correlated with DOC. In addition to the above, plant cover was also negatively correlated with DOC and GWC, but positively correlated with soil pH.
Correlations conducted within amendment treatments demonstrated that these relationships between environmental and biological variables were influenced by and subsequently changed in response to amendment (Tables S9–S12). In particular, the relationships between soil efflux and plant cover were not significant in unamended control or surface-applied woodchip plots, but significantly positively correlated when woodchips or biochar were incorporated into the soil. Furthermore, soil efflux and GMEA were not significantly correlated in unamended control and incorporated biochar plots. However, there was a significant positive relationship between soil efflux and GMEA for both surface-applied and incorporated woodchip amendments.

Univariate linear regression demonstrated that in unamended control plots, neither plant cover nor GMEA explain the variation observed in soil efflux (Fig. 4A). However, GMEA was a significant predictor of soil efflux in surface-amended plots (Fig. 4B). Both plant cover and GMEA were significant predictors of soil efflux in incorporated woodchip plots and only plant cover was a significant predictor of soil efflux in incorporated biochar plots (Fig. 4C & 4D). Multiple linear regression analysis was used to better understand the predictive power and relative importance of plant cover and exoenzyme activities on observed soil efflux (Table 2). Within each amendment treatment, plant cover and GMEA were used to explain the variance observed in soil efflux. The model was significant for the surface

![Figure 3. Principal component analysis (PCA) biplot results. The scatter plot matrix shows the first two components from the PCA grouped by amendment treatment.](image)

**Table 1.** Pearson’s correlation coefficients for biological and soil C and N across all soil types, amendments, and sample dates. Significant correlations are highlighted in italic bold (p ≤ 0.09) and bold (p ≤ 0.05) in accordance with a Pearson’s paired sample association test.

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<th>N-EE</th>
<th>P-EE</th>
<th>GMEA</th>
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<td>-0.35</td>
<td>0.09</td>
<td>-0.15</td>
<td>1.00</td>
</tr>
</tbody>
</table>
woodchip, incorporated woodchip, and incorporated biochar amendment, but not significant for the unamended controls (Table 2). A greater proportion of explained variance was attributed to plant cover for the surface woodchip and incorporated biochar treatment. Conversely, GMEA had relatively greater importance in predicting soil efflux when woodchips were incorporated.

**Discussion**

Long-term restoration strategies require an understanding of the complexity and variability of plant–soil interactions (Eviner & Hawkes 2008). This field experiment was designed to test the responses and interactions of native plant establishment, soil C, N, and efflux, along with microbial exoenzyme activities and biomass to woody amendments in a semiarid grassland. We measured biological activities following seasonal precipitation events to capture their responses to pulse-driven moisture availability (Jenerette et al. 2008). As we expected, we found that plant cover, soil efflux, and microbial biomass and exoenzyme activities were the most responsive to the larger pulse event of the summer monsoons. Conversely, dissolved soil C and N contents were insensitive to the differences between seasonal precipitation events and instead accumulated over time. The relative accumulation in C was greater than N resulting in higher DOC:TDN over time, which is discussed further below.

We tested two predominate soil types in our study area to aid in the generalization of this work and applicability to other semiarid ecosystems. Only microbial N and P exoenzyme activities were sensitive to soil type, with less activity in Hathaway soil that may be explained by significantly greater drying in Hathaway compared to Chiricahua soils (Rasmussen et al. 2015). Given that exoenzymes are active in water-films on soil particles, greater soil drying in the Hathaway soils likely limits exoenzyme activities.

We found positive, neutral, and negative effects of amendments on plant cover and microbial activities based on amendment type (woodchip or biochar) and its location on the soil surface or incorporated into soil. The surface application of woodchips to soil resulted in the greatest increase in microbial activities (C and N-EE) and SOC; however, it did not significantly aid revegetation compared to nonamended controls. Incorporating woodchips suppressed vegetation and stimulated soil carbon loss via heterotrophic soil efflux while also enhancing production of DOC. Biochar had an overall neutral effect on plant cover, microbial

**Table 2.** Multiple linear regression analyses for soil efflux using plant cover and GMEA as explanatory variables. Significant model p values are highlighted bold (p ≤ 0.05).

<table>
<thead>
<tr>
<th>Amendment Type</th>
<th>Statistic</th>
<th>SE</th>
<th>Relative Importance</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unamended control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant cover</td>
<td>2.30</td>
<td>0.12</td>
<td>0.479</td>
<td>0.07</td>
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<tr>
<td>GMEA</td>
<td>−0.85</td>
<td>0.82</td>
<td>0.060</td>
<td>0.44</td>
</tr>
<tr>
<td>Model p = 0.14; F statistic = 2.93 on 2 and 5 df</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface-applied woodchips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant cover</td>
<td>0.46</td>
<td>0.05</td>
<td>0.176</td>
<td>0.66</td>
</tr>
<tr>
<td>GMEA</td>
<td>1.65</td>
<td>0.31</td>
<td>0.317</td>
<td>0.13</td>
</tr>
<tr>
<td>Model p = 0.05; R² = 0.49; F statistic = 4.376 on 2 and 9 df</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorporated woodchips</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant cover</td>
<td>2.20</td>
<td>0.09</td>
<td>0.253</td>
<td>0.05</td>
</tr>
<tr>
<td>GMEA</td>
<td>3.60</td>
<td>0.28</td>
<td>0.463</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Model p &lt; 0.001; R² = 0.71; F statistic = 13.82 on 2 and 11 df</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incorporated biochar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant cover</td>
<td>4.25</td>
<td>0.06</td>
<td>0.679</td>
<td>0.004</td>
</tr>
<tr>
<td>GMEA</td>
<td>−0.85</td>
<td>0.27</td>
<td>0.079</td>
<td>0.423</td>
</tr>
<tr>
<td>Model p &lt; 0.007; R² = 0.66; F statistic = 10.95 on 2 and 7 df</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
activities, and soil carbon over the 22 months of this field study. In general, the application of woody amendments was found to leave a signal in soils via DOM contents and stoichiometry as well as in microbial biomass C, which distinguished the soils with woody amendments from those with incorporated biochar or no amendment.

Implications for Revegetation

While woodchips were expected to enhance plant establishment by regulating soil moisture availability (Lakatos et al. 2000; Hueso-González et al. 2014; Hueso-González et al. 2018), surface-applied woodchips had neutral effects on plant cover and incorporating woodchips significantly reduced plant cover. The decrease in plant cover in the incorporated woodchip treatment suggests that application location is a significant factor when reseeding disturbed soils. This negative effect of incorporated woodchips on plant cover is corroborated by growth chamber and greenhouse experiments using the same soil types and plant species (Gebhardt et al. 2017; Fehmi et al. this issue). GWC in surface woodchip or incorporated woodchip soils was not significantly different from control plots in either this field study or a greenhouse study (Fig. S4; Fehmi et al. this issue), and therefore, soil moisture cannot account for the treatment differences in plant cover. Additionally, neither the surface nor the incorporated woodchip treatments promoted plant cover following the smaller winter rains, which would have been expected if woodchip application buffered against soil moisture loss as has been observed in other studies (Hatfield et al. 2001; dos Santos et al. 2010).

Biochar did not increase plant cover and we did not observe changes in soil water content. These results are in contrast to results from agriculture and greenhouse experiments using similar biochar and pyrolytic conditions (Blackwell et al. 2010; Lentz & Ippolito 2012). While Gebhardt et al. (2017) and Fehmi et al. (this issue) both found increased soil water content in biochar-amended soils, plant biomass was not significantly different than the nonamended control treatment. There is evidence from other studies that lignin-rich woody biochar may result in nutrient limitation surpassing water availability as a more regulating factor controlling plant growth (reviewed in Gul et al. 2015). For example, microbial communities relying on substrate with a greater than 25:1 ratio of C:N, which is the case in this experiment (138:1 and 69:0 for woodchip and biochar amendments, respectively), assimilate inorganic N to meet their metabolic needs, immobilizing soil N, and thus making it unavailable to plants (Booth et al. 2005; Gebhardt et al. 2017). Furthermore, biochar itself has a high cation exchange capacity and may adsorb inorganic nutrient cations (Gundale & DeLuca 2006). Therefore, both nutrient and soil moisture availability must be considered when interpreting plant responses to biochar amendments.

We expected amendments would have indirect benefits to plant establishment and growth; however, we found the opposite. At best, the amendments had no effect on plant cover. Incorporating woodchips had an adverse effect and seemingly inhibited initial plant growth resulting in low plant cover. We expected that effects of biochar might not be resolved over the duration of the current analysis; however, given unfavorable moisture dynamics to stimulate biotic or abiotic oxidation, biochar may continue to have a neutral effect on plant cover. Regardless, these amendments alone are not a recommended practice for restoration efforts aimed at promoting plant cover quickly.

Implications for Soil C

An overall divergence between the woodchip treatments (surface and incorporated) and control and incorporated biochar was driven by DOC, MBC, and the DOC:TDN ratio. This suggests that regardless of application location, woodchips influence soil C dynamics and microbial growth similarly. However, the observed increase in soil efflux in the incorporated woodchip treatment combined with low plant growth not similarly observed in the surface treatment suggests that application location is a significant factor for biological processes. Similar results regarding the importance of amendment location on microbial responses have been observed in arid ecosystems (Biederman & Whisenant 2011; Li et al. 2018). Further analysis is required to better understand which pool of soil C is being used by the microbial community and released from the soil, the woodchip C or preexisting soil C, as well as to better understand what this would mean for the long-term consequences for soil C if the pattern of high soil efflux and low plant cover, reducing the plant-pump of chemically diverse C into soil, continues.

Soil organic C was greater in the surface woodchip treatment and soil DOC was greater in both surface and incorporated woodchip amendments compared to controls. Woody debris and amendments can be a significant source of soil DOC (Hafner et al. 2005), which is composed of mobile, microbialy available, and chemically complex C substrates produced by leaching of plant biomass and root exudates as well as microbial activities (McDowell & Likens 1988; Christ & David 1996; Dakora & Phillips 2002; Blankinship & Schimel 2018). Therefore, surface-applied woodchips can be a direct source of soil C. In our study, C- and N-acquiring exoenzyme activities also increased with surface woodchips, which may have been in response to, or physically contributed to, the observed increases in DOC. In contrast, incorporating woodchips into soil did not significantly enhance SOC; however, soil DOC increased, which subsequently increased the DOC:TDN ratio. This shift in DOM stoichiometry may signal N-limitation or lack of N substrate and thus limited production of exoenzymes (e.g. Allison & Vitousek 2005) resulting in the observed low activities. These differences between surface and incorporated amendments may have also been influenced by plant cover, which was significantly lower in incorporated woodchip plots. Plant roots are a source of soil nutrients, and the reduced vegetation when woodchips were incorporated may also contribute to the observed DOC:TDN dynamics. Conversely, the presence of vegetation in the surface-applied woodchip treatment may contribute to the enhanced exoenzyme activities.

Biochar is commonly used to enhance nutrients and sequester C in soils (Lehmann et al. 2006; Xu et al. 2013), though its effects on microbial activities and soil biogeochemistry are highly variable and dependent on regional factors, biochar type,
Woodchip and biochar amendments

and addition rate (Wang et al. 2015). After 22 months of field conditions in semiarid ecosystem soils, biochar had no significant effects on soil efflux, DOC, TN, microbial biomass C, or exoenzyme activities. Similarly, Sun et al. (2014) found that soil efflux and exoenzyme activities were not influenced by woody biochar additions after one year of experimental treatment. It is possible that the duration of these studies might not have been sufficient to observe the effects of biochar given its slow decomposition rate (Steinbeiss et al. 2009), which is likely exacerbated by water limitation in semiarid ecosystems. Alternatively, or additively, the size of the char may have inhibited microbial interactions. The biochar used in this experiment ranged in sizes on the centimeter scale, which limits available sorption sites and surface area for microbial colonization and activities. However, pulverization of biochar materials may not always be feasible, therefore it is important to understand further how larger particle size biochar positively or negatively affect soil microbial communities, and in turn, how they interact with biochar.

Through adding coarse woody and biochar amendments a significant amount of carbon was input directly to soils where materials decompose slowly given low and only seasonally available moisture. Further analysis over time is required to better understand the decomposition rate of these materials and their influence on long-term soil C stocks.

Soil Efflux and Plant–Microbe Interactions in Response to Amendments

Incorporated woodchips inhibited plant cover yet promoted the greatest soil efflux of all the treatments. Soil CO2 efflux is the combined respiration produced by plant roots and heterotrophic microbes. Therefore, changes or lack thereof in one or both microbial and plant activities paired with soil CO2 efflux observations provides insight into their interactions. Microbial biomass C was not influenced by incorporating woodchips and therefore microbial growth is unlikely to account for observed increased soil efflux.

While soil efflux was overall positively correlated with plant cover and exoenzyme activities, the amendment treatments influenced the relationship between soil efflux, plant cover, and microbial activities. Notably, in the unamended control plots, soil efflux was not dependent on plant cover or microbial activities. Conversely, when surface woodchips were added to the plots soil efflux was significantly positively correlated to exoenzyme activities. Furthermore, when woodchips were incorporated into the plots, both plant cover and exoenzyme activities were significantly drivers of soil efflux and together were able to predict 70% of the variation in soil efflux.

One possible explanation for the unexpected enhanced soil efflux when woodchips were incorporated is that the changes in soil DOC/TKN resulting from incorporating woodchips that reduce microbial carbon use efficiency. Carbon may be lost through respiration from overflow metabolism—defined as the decoupling of catabolism from microbial growth in nutrient limiting conditions (Schimel & Weintraub 2003; Chapin et al. 2011). Woody amendments are a lignin-rich, high C:N source of C for soil microbes and require more energy to break down than simple labile compounds (Davidson & Janssens 2006). Under N limitation and when faced with low quality substrate, microbial decomposition and C use efficiency decline (De Graaff et al. 2010; Manzoni et al. 2012). Evidence of this was observed in the significant increase in soil DOC:TN when woodchips were incorporated. This phenomenon has also been demonstrated in decomposition models where adding C to an N-limited system is shown to increase respiration as C is directed to waste respiration because microbial growth is limited by N (Schimel & Weintraub 2003). Furthermore, the reduced amount of vegetation and therefore active roots in this treatment likely also reduced nutrient input into the soil as plant roots are a significant source of nutrients and exert a strong influence on the soil microbial community (Haichar et al. 2014). These restoration strategies, while overall ineffective in the short-term efforts towards revegetation of heavily disturbed soils, provided useful insight into microbial responses and plant microbe interactions. Further observation over several years will provide additional insight into whether these treatments may influence soil seed banks, long-term soil carbon dynamics, and microbial activities.

Acknowledgments

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Woodchip and biochar amendments


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Supporting Information

The following information may be found in the online version of this article:

Table S1. Soil characteristics for Chiricahua and Hathaway experimental soils analyzed at the start of field experiment in June 2013.

Table S2. Amendment characteristics for woodchip and biochar materials added to soils.

Table S3. Amount of amendment added to plots by soil type in grams to achieve 4% by weight application rate.

Table S4. Exoenzyme activities measured.

Table S5. MANOVA of the response variables plant cover, soil efflux, MBC, SOC, DON, and DOC:DON.

Table S6. MANOVA of soil CEE, NEE, PEE, and DON.

Table S7. Means (standard error of the means) for soil exoenzyme activities (BG, AG, CB, XYL, NAG, LAP and PHOS) calculated from untransformed data.

Table S8. PCA loadings used for bigplot.

Table S9. Pearson’s correlation coefficients (r) for biological and soil C and N within unamended control plots.

Table S10. Pearson’s correlation coefficients (r) for biological and soil C and N within surface woodchip amended plots.

Table S11. Pearson’s correlation coefficients (r) for biological and soil C and N within incorporated woodchip amended plots.

Table S12. Pearson’s correlation coefficients (r) for biological and soil C and N within incorporated biochar amended plots.

Figure S1. Total monthly precipitation (A) and average monthly air temperatures (B) for the experimental site.

Figure S2. Soil C, N, and P exoenzyme activities and microbial biomass C by soil type (Hathaway and Chiricahua).

Figure S3. Soil C, N, and P exoenzyme activities and microbial biomass C by amendment.

Figure S4. Means ± SE (n = 4) for gravimetric water content across amendments.

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