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Soil properties explain changes in soil microbial enzyme activity with depth in a piñon-juniper woodland

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SOIL PROPERTIES EXPLAIN CHANGES IN SOIL MICROBIAL ENZYME ACTIVITY WITH DEPTH IN A PIÑON-JUNIPER WOODLAND

by

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ABSTRACT

Semi-arid ecosystems play a major role in the global carbon cycle. These ecosystems, including piñon-juniper (PJ) woodlands, are experiencing extreme drought. As such, it is vital to characterize both above and below-ground processes in these systems in order to understand their vulnerability to future drought and other changes in climate.

Soils microbial communities play a critical role in nutrient cycles, as well as carbon storage, within PJ woodlands. More specifically, microbes aid the decomposition and mineralization of key nutrients, including carbon, nitrogen and phosphorus, through the use of extracellular enzymes. Measuring microbial enzyme activity, therefore, can provide insight into how nutrients transform within an ecosystem. I measured enzyme activity in both soils and roots collected at different depths in order to investigate patterns of enzyme activity with depth, as well as to determine if there are differences between piñon and juniper root enzyme activities. I also measured various soil and root chemical properties to determine which factors, if any, dictate these patterns. Specifically, we measured β-1,4-glucosidase (BG), which helps release carbon from cellulose, β-1,4-N-acetylglucosaminidase (NAG), which helps release nitrogen from

chitin and bacterial cell walls, and acid phosphatase (AP), which helps release phosphorus from nucleic acids and cell membranes.

I tested three hypotheses: 1) soil microbial enzyme activity should increase with canopy because of greater organic matter and rhizosphere inputs; 2) the petro-calcic horizon will dictate patterns in enzyme activity, since the horizon represents a physical barrier between the surface and subsurface layers that directly influences nutrient cycling and nutrient concentrations; 3) and because microbial enzyme activity reflects the interaction between microbial nutrient demand and nutrient availability, enzyme activity should reflect changes in nutrient limitation throughout the soil profile.

Our results indicated that patterns in soil microbial BG, AP and NAG activities were dictated by changes in both percent soil organic carbon and percent total nitrogen with depth. Also, AP activity was higher compared to both BG and NAG, indicating that PJ woodlands, like other semi-arid biomes, are more phosphorus limited. Unfortunately, inferences about the differences between piñon and juniper root enzyme activities could not be determined. Measuring microbial enzyme activity throughout the whole profile is important as it can provide insights into both microbial processes and nutrient cycling within these rapidly changing ecosystems.

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Introduction

Globally, terrestrial ecosystems are important carbon sinks, although there is variability in the strength of the sink depending on the ecosystem. Climate models indicate that semi-arid regions are responsible for most of the inter-annual variability in atmospheric CO_2 concentration (Poulter et al. 2014, Ahlström et al. 2015). One reason for the extreme variability of semi-arid biomes is they routinely experience a range of disturbances including drought (IPCC, 2007), climate-driven mortality (Allen et al. 2010; 2015), desertification (Schlesinger, 2000), woody encroachment (Knapp et al. 2008), grazing, fire, and land use (Reynolds et al. 2007; Campbell et al. 1997). Soils in particular, are critical components of the carbon budget in arid and semiarid soils (Evans et al. 2017; Poulter et al. 2014). Semi-arid soils are typically low in resource availability, which makes them more responsive to small changes in resource availability, and therefore, more vulnerable to drivers of global change than more mesic systems (Evans et al. 2017). Increasing our understanding of soil carbon and nutrient cycling in semi-arid biomes, therefore, is critical to understanding how resilient these ecosystems may be to future change.

Soil water-holding capacity, the spatial distribution of soil carbon, water and nutrient availability, and a functional understanding of the microbial community, all have potential consequences for understanding the vulnerability of these biomes (Evans et al. 2017). For example, soil water-holding capacity can influence resilience of semi-arid biomes, in that vegetation can maintain productivity during dry periods if there is an adequate supply of water in the soil accessible to plants (Peterman et al. 2013). Soil organic matter, soil texture and organic

carbon content all increase soil water-holding capacity in these soils (Hudson, 1994; Rawls et al. 2003). Vegetation cover and composition directly influence the spatial distribution of nutrients and water, via differences in litter quality and growth form (Evans et al. 2017). Changes in vegetation composition in semi-arid biomes due to woody encroachment have been associated with decreases in soil pH and increases in total carbon, nitrogen and exchangeable soil calcium (Eldridge et al. 2011). Shifts in functional microbial communities due to environmental stress alters biogeochemical processes within ecosystems, as different groups of microbes perform different ecosystem functions (Schimel et al. 2007).

Our understanding of microbial communities in particular, including the important factors that regulate them, and the functional roles they play in semi-arid ecosystems has improved in recent years (Evans et al. 2017). The specific plant community present (Dean et al. 2015; Evans et al. 2017), water availability (Manzoni et al. 2012), timing in water availability (Austin et al., 2004), disturbance, mortality of dominant species (Warnock et al 2016), and soil carbon, nitrogen and pH (Sinsabaugh & Follstad Shah 2012; Kivlin & Treseder 2014) all influence microbial activity in semi-arid biomes. In addition, plant community structure can be an important determinant, as resource islands often exist under the canopies of dominant plant species compared to inter-canopy areas (Bolton et al. 1993). Greater organic matter inputs under the canopy typically enhance microbial biomass and the resulting biological activity (Bolton et al. 1993).

Our goal for this study was to understand soil microbial enzyme activity in one semi-arid biome, piñon-juniper (PJ) woodlands. (PJ) woodlands are the third largest biome in the southwestern United States, and are currently experiencing multiple drivers of mortality, including insect outbreaks and sustained drought (West 1999; Peterman et al. 2013). We focused

specifically on quantifying how soil microbial enzyme activity varied with soil depth. While enzyme activity has been studied in soils globally (Sinsabaugh et al. 2008), very few studies have incorporated depth. It is particularly important to study changes in microbial activity with depth in many semi-arid biomes due to the presence of a well-developed petrocalcic (caliche) horizon, that forms via the precipitation of carbonates in the soil (Hennessy et al. 1983). Caliche can absorb large amounts of water, although water does not percolate through very easily (Hennessy et al. 1983; Duniway et al. 2007). In many sites, a well-developed caliche layer can be a barrier to root growth, although there is evidence of both tree and shrub roots growing throughout this layer as well (Gibbens & Lenz, 2001; Pangle et al., 2012). Cunningham & Burk (1973) conducted a study in New Mexico and found when there was little precipitation, the caliche layer enhanced the water status of creosote. Considering that caliche might act as water storage and that plant roots grow with depth, it is vital to characterize belowground processes throughout the entire soil profile. If microbial activity is relatively high within and below the caliche layers, this might indicate that roots growing through the caliche are actively acquiring nutrients from deeper layers in the soil profile. We tested three hypotheses: 1) soil microbial enzyme activity should increase with canopy density due to greater organic matter and rhizosphere inputs; 2) the petro-calcic horizon will dictate patterns in enzyme activity, as the horizon represents a physical barrier between the surface and subsurface layers that directly affects nutrient cycling and nutrient concentrations; 3) and because microbial enzyme activity reflects the interaction between microbial nutrient demand and nutrient availability, enzyme activity should reflect changes in nutrient limitation throughout the soil profile.

Methods

2.1 Site Description (Study site and sample collection)

Our field site is 35 miles east of Las Vegas, New Mexico (35°63'N, 104°64'W), in a piñon-juniper woodland. The 30-year-site average (1981-2010) annual precipitation is 458.7 mm and the 30-year average temperature is 10.9 °C (PRISM, Climate Group, Oregon State University; http://prism.oregonstate.edu). During the sampling period (March, 2017), total precipitation was 16 mm and average temperature was 9.5 °C, which compares to 30-year-site average of 22 mm precipitation in March and 5.7 °C average temperature (PRISM). The overstory is entirely piñon (*Pinus edulis*) and juniper (*Juniperus monosperma*). Mean basal area of piñon and juniper is 10.9 ± 0.63 m^2/ha and 10.8 ± 1.17 m^2/ha, respectively. Piñon tree height varied from 1.0 – 7.1 m (mean 4.1 ± 0.19 m) and juniper tree height varied from 1.0 – 6.0 m (mean 3.5 ± 0.27 m). *Opuntia* species, *Quercus berberidifolia*, and *Bouteloua gracilis* make up the bulk of the understory at the site.

2.2 Soil and root sampling for enzyme activity

In March 2017, we used a backhoe to dig three 10-meter-long, 1.5-meter-deep, trenches, that started in the canopy on one end and extended into the intercanopy. We collected soil samples from the trenches on March 13 and 14, at meter 1, 5 and 9 along the length of each trench to capture changes in canopy cover. At each of those locations, we collected ~10 g of soil at 10, 30, 50, 100 and 140 cm depth from the surface, and stored the samples in Ziploc bags in a freezer until processed.

Official soil classifications in the three pits were done by NRCS. We followed the hydrometer protocol for particle size analysis on the whole fine earth fraction in the soil layers

identified by NRCS (Burt, 2009) (Table 1). In all three pits, sand content decreased and clay and silt content increased with depth.

We collected piñon and juniper roots at two depth intervals, 0-10 cm and 30-50 cm, at meters 1, 5 and 9, and stored the roots in Ziploc bags in a freezer until they were processed.

Table 1. Soil particle analysis and description for soils from the three trenches.

Trench	Depth(cm)	Sand	Clay	Silt	Texture
1	0-18	0.4	0.26	0.34	loam
	18-28	0.35	0.35	0.3	clay loam
	28-43	0.35	0.39	0.26	clay loam
	43-68	0.29	0.33	0.38	clay loam
	68-140	0.36	0.25	0.39	loam
2	0-8	0.36	0.26	0.38	loam
	8-28	0.38	0.31	0.31	clay loam
	28-61	0.32	0.36	0.32	clay loam
3	0-13	0.46	0.29	0.25	sandy clay loam
	13-46	0.42	0.29	0.29	clay loam
	46-60	0.16	0.45	0.39	loam

2.3 Root distribution and density

We also collected roots to quantify root distribution with depth in all three trenches. We used a soil knife and/or pick axe to cut three 10 x 10 x 10 cm^3 soil blocks in 10 cm increments from the surface to 150 cm, in every meter of each 10 m long trench. We sieved the soil blocks in the field and stored all the roots in each soil block in labeled Ziploc bags. In the laboratory, we sorted these roots into four diameter size classes (<0.8 mm; 0.8-5 mm; 5-20 mm; and > 20 mm). We sorted roots greater than 0.8 mm into species (piñon or juniper). All roots < 5 mm in diameter were scanned and used to estimate root length with WinRHIZO (Regent Instruments Inc., Quebec, CA) (Schwinning et al., in prep). We used only the root densities from the trench

locations/depths samples used for microbial enzyme activity in this analysis. Fine root density (mm/cm^3) was calculated for fine roots only (2mm in length or less), as fine roots are the most active in acquiring nutrients and water.

We quantified above ground basal area for all trees within a 3-meter radius of each sampling location to estimate canopy cover along the trench.

2.4 Soil chemical analyses

We modified the protocol of Walthert et al. (2010) to measure total organic carbon and total nitrogen after removal of inorganic carbon from our samples. We weighed approximately 25 mg of oven dried ground soil (24 hours at 60 °C) into silver capsules and placed the capsules in a titer plate. We then added 50 μL of 1% HCl solution to each capsule before we placed the titer plate in a desiccator and exposed the samples to vapor from 37% HCl for eight hours, and then 32% HCl for 24 hours. After adding 50 μL of deionized water to each capsule, we exposed the samples again to 37% HCl for an additional 8 hours. The titer plate was placed in a vacuumized desiccator for 24 hours, and then a vacuum oven at 200-300 hPa at 35-40 °C for 3-7 days. The packed capsules were then wrapped in larger silver capsules to prevent loss of sample before they were measured for percent carbon and nitrogen using a CN analyzer (NC2100 Elemental Analyzer, ThermoQuest Italia S.p.A., Rodano, Italy).

We measured soil pH by adding 2 grams of soil and 20 mL of distilled water to Nalgene bottles, and after letting the suspensions equilibrate for 2 hours, and took pH readings using a pH meter (Hanna instruments.)

2.5 Enzyme Assays

2.5.1 *Soil microbial enzyme activity*

Soils and roots were assayed for the potential activities of β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG) and acid phosphatase (AP). BG helps break down cellulose, the most abundant organic carbon molecule; NAG helps break down chitin and bacterial cell walls, sources of nitrogen in the soil; and AP helps release phosphorus from nucleic acid and cell membranes, an important step for phosphorus cycling (Sinsabaugh & Follstad Shah, 2012) (Table 2). For each of the 15 soil blocks analyzed in each trench (locations described above), we added 1.0g of fresh soil to 50 mL of 50 mM sodium bicarbonate (pH 8) in 125mL bottles. After homogenizing each soil-buffer solution for 60 seconds using a Brinckman Polytron pt3000, we filled the bottle with an additional 75 mL of buffer and pipetted 200 μL aliquots into the 96-well black microplates (8 rows, 12 columns), for both the negative control and assay plate. The negative control plate contained 200 μL soil suspension + 50 μL buffer in every well. The assay plates contained a blank column where every well was filled with only 250 μL buffer; a standard reference column where every well contained 200 μL buffer + 50 μL 4-methylumbelliferone (standard); a substrate reference column where every well contained 200 μL buffer + 50 μL substrate. Three of the columns in the assay plate were used as quench control to ensure that the standard worked and contained 200 μL of soil suspension + 50 μL standard. Six of the columns in the assay plate were used for the sample assays and contained 200 μL of soil suspension + 50 μL substrate. After enzyme-dependent incubation time, the microplates were measured using a BioTek Synergy H1 Hybrid Reader. We calculated enzyme activity as nmol h^{-1} g^{-1} substrate and

express it in two ways: 1) per dry weight of each soil sample as nmol h^{-1} g^{-1} dry mass and 2) per percent soil organic carbon of each soil sample as nmol h^{-1} g^{-1} SOC.

2.5.2 *Root enzyme activity*

We ran a second series of enzyme assays to measure activities of AP, BG and NAG from collected piñon and juniper roots (both piñon and juniper roots combined). In place of 1.0-gram soil, 0.5 grams of ground roots were added to empty 125 mL bottles, and then the same protocol was conducted as the soil assays. Root enzyme activity was also expressed per dry weight of each root sample (nmol h^{-1} g^{-1} dry mass) and as a fraction of soil organic carbon (nmol h^{-1} g^{-1} SOC).

Table 2. Summary of enzymes studied and their functions.

Enzyme	**Substrate**	**Enzyme function**
Acid phosphatase (AP)	4-MUB-phosphate	Releases phosphorus from nucleic acid, cell membranes
β-1, 4-Glucosidase (BG)	4-MUB-β-D-glucoside	Releases carbon from cellulose
N-acetyl-glucosaminidase (NAG)	4-MUB-N-acetyl-β-D-glucosaminide	Release nitrogen from chitin, bacterial cell walls

We also calculated BG:AP and BG:NAG enzyme ratios to make inferences about nutrient distribution within the site; enzyme ratios are considered to be a link between microbial ecology and ecological stoichiometry (Sinsabaugh et al. 2008).

2.6 Data Analysis

Soil enzyme activity per dry mass of the soil (nmol h^{-1} g^{-1}) was used to compare differences in AP, BG and NAG activities and correlate enzyme activity with soil chemical data. Soil enzyme activity calculated as nmol $h^{-1}g^{-1}$ SOC was used to compare activities in relation to quality and quantity of soil organic carbon with soil depth. Root enzyme activity (nmol h^{-1} g^{-1}) was used to directly compare differences between AP, BG and NAG activities and converted to activity nmol h^{-1} g^{-1} SOC to directly compare root and soil enzyme activity per unit carbon. Both soil and root enzyme data were log-transformed prior to data analyses to meet assumptions of normality.

All statistical analyses were performed using R (version 3.3.2). Soil enzyme activity was averaged for each depth across the three trenches, as the activity didn't vary significantly between the trenches.

To assess which factors, if any, help explain patterns of soil enzyme activity with depth, the relationship between log-transformed soil enzyme activity and percent soil organic carbon, percent total nitrogen, and fine root density was examined. The relationship between log-transformed root enzyme activity against root percent carbon and percent nitrogen was also explored. Best fit was assessed using AIC, and statistical significance was determined at the $\alpha = 0.05$ level.

Fine root density (mm/cm^3) was calculated for fine roots only (2mm in length or less), as fine roots are the most active in acquiring nutrients and water.

Due to small sample size, a randomization wrapper was used before running any models, which involved running an analysis on a random sample of all possible permutations of the soil enzyme data to determine the distribution (Cassell 2002). Linear mixed effects models were fit to

the data using the R package lme4. The models were used to determine the effects of enzyme type, soil depth, soil organic carbon, total nitrogen, pH, fine root density and basal area on soil enzyme activity. Enzyme type, soil depth, soil organic carbon, total nitrogen, pH fine root density and basal area were treated as fixed effects, while trench was treated as a random effect. Models were compared using AIC. We also assessed r-squared values to determine how well the models fit.

Results

3.1 Soil physical properties

Percent soil organic carbon and percent total nitrogen did not vary significantly across the three trenches, but both decreased significantly with soil depth (Figure 1A and 1B, Table 3). Depth explained 80% of the variance in percent carbon and explained 75% of the variance in percent nitrogen. Neither C:N nor pH significantly decreased with depth in any trench. pH was significantly different in all three trenches, likely due to varying depth of the caliche layer (70 cm trench 1, 40 cm trench 2, and 30 cm in trench 3) (Figure 2; Table 3).

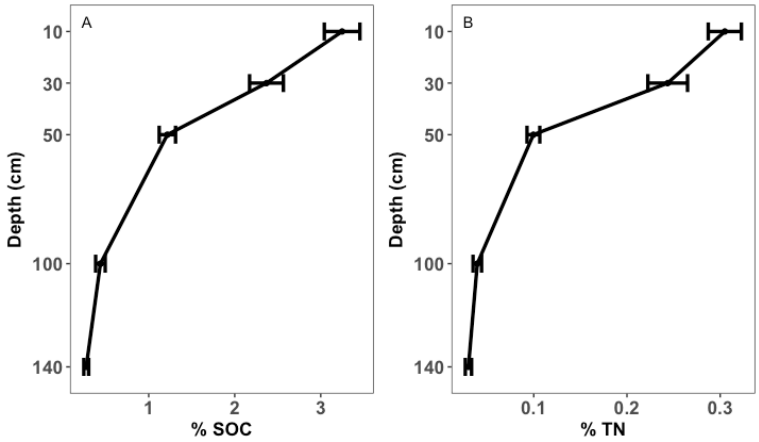

Figure 1. Decrease in A) average percent soil organic carbon and B) average percent total nitrogen with soil depth. % soil organic carbon and % total nitrogen values were averaged across the three trenches, for each depth.

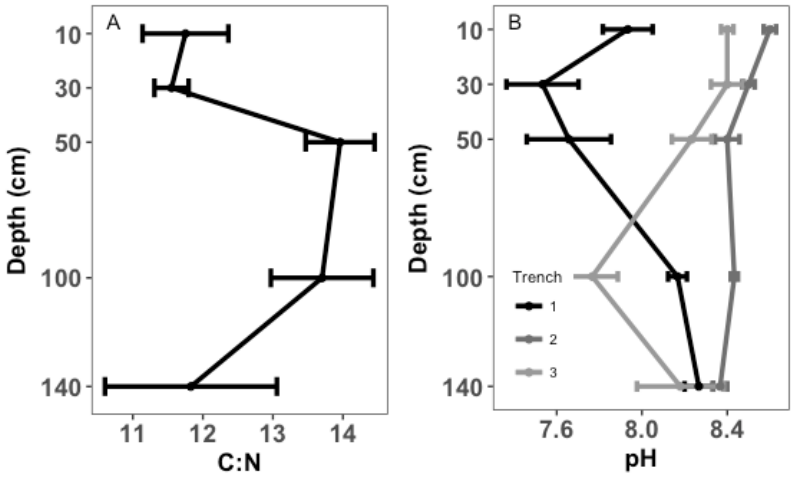

Figure 2. Change in A) average C:N ratio and B) average pH with soil depth. C:N values were averaged across the three trenches, for each depth. pH values were averaged across the depths, for each trench.

Table 3. Regression statistics relating soil properties to soil depth and/or trench.
* indicates significance at the p< 0.05 level.

Response	**Effect**	**p-value**
% SOC	Depth	0.024*
% TN	Depth	0.035*
C:N	Depth	0.776
pH	Depth	0.093
	Trench	0.028*
	Depth*Trench	0.072

3.2 Fine Root Density

Piñon fine roots only, juniper fine roots only and total fine root density, significantly decreased with soil depth, but did not significantly differ between the open or more tree clustered ends of the trenches (Figure 3; Table 4). When fine root density was averaged across the open vs.

clustered locations and trenches, the strength of the relationship between average fine root density and depth varied by species: piñon R^2 value = 0.72; juniper R^2 value = 0.80; and total R^2 value = 0.75.

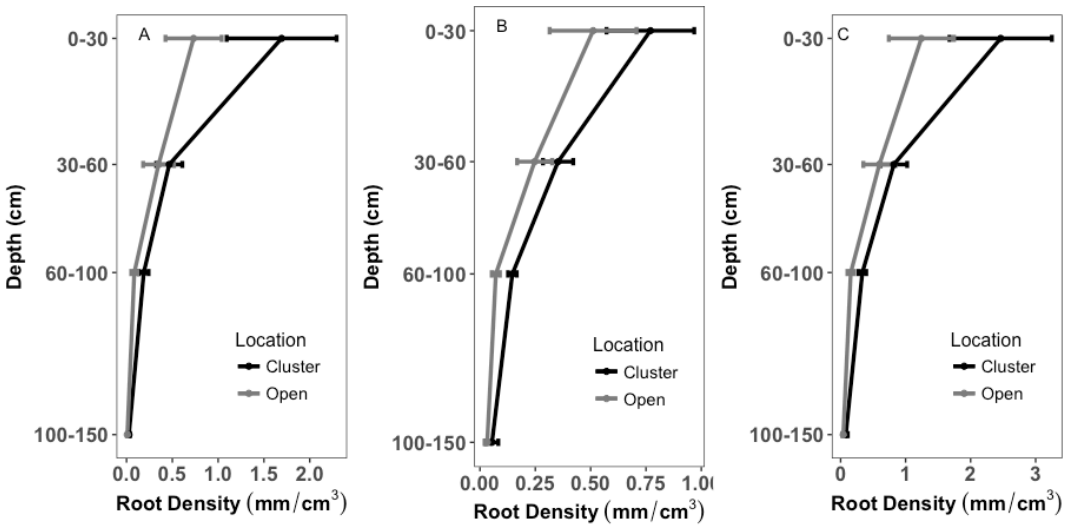

Figure 3. Decrease in average A) piñon fine root density, B) juniper fine root density and C) total fine root density with soil depth. Values were averaged across the three trenches, for each depth and location. Total fine root density was calculated by adding piñon and juniper fine root density together.

Table 4. ANOVA statistics comparing average fine root density and soil depth and location

Response	**Effect**	**Degrees of freedom**	**Sum of squares**	**F value**	**P value**
Piñon	Depth	1	1.33001	12.7602	0.023*
	Location	1	0.35354	3.3918	0.139
	Depth*Location	1	0.19300	1.8517	0.245
Juniper	Depth	1	0.26585	18.6455	0.012*
	Location	1	0.03366	2.3604	0.199
	Depth*Location	1	0.01350	0.9469	0.386
Total	Depth	1	2.7851	14.6481	0.019*
	Location	1	0.6053	3.1838	0.149
	Depth*Location	1	0.3086	1.6230	0.272

3.3 Soil microbial enzyme activity

Activity for all three enzymes significantly decreased with soil depth when normalized by dry mass (Figure 4, Table 5). AP activity was the most strongly correlated with depth ($R^2 = 0.85$), followed by BG ($R^2 = 0.81$) and NAG ($R^2 = 0.77$). On average, AP, NAG and BG activity was 75%, 51% and 71% greater in soils collected at 10 cm compared to soils collected at 140 cm, respectively. If we compare the slopes of Ln (Activity) versus depth, AP activity had a lesser slope (m = -0.018) than NAG (m = -0.009) and BG (m = -0.012), indicating that AP activity decreased with soil depth faster than the other two enzymes.

NAG activity, when normalized to soil organic carbon, was the most strongly correlated with depth (R^2 value = 0.78), followed by AP ($R^2 = 0.66$) and BG ($R^2 = 0.55$). On average, AP, NAG and BG activity per g SOC was 13%, 15% and 14% greater in soils collected at 10 cm compared to soils collected at 140 cm, respectively. If we look at the slopes of Ln (Activity) versus depth, AP activity normalized by percent soil organic carbon also had a lesser slope (m = -0.009) compared to NAG (m = -0.007) and BG (m = -0.005). The more rapid decline of AP with depth, compared to either NAG or BG was consistent regardless of whether enzyme activity was normalized by dry mass or percent soil organic carbon.

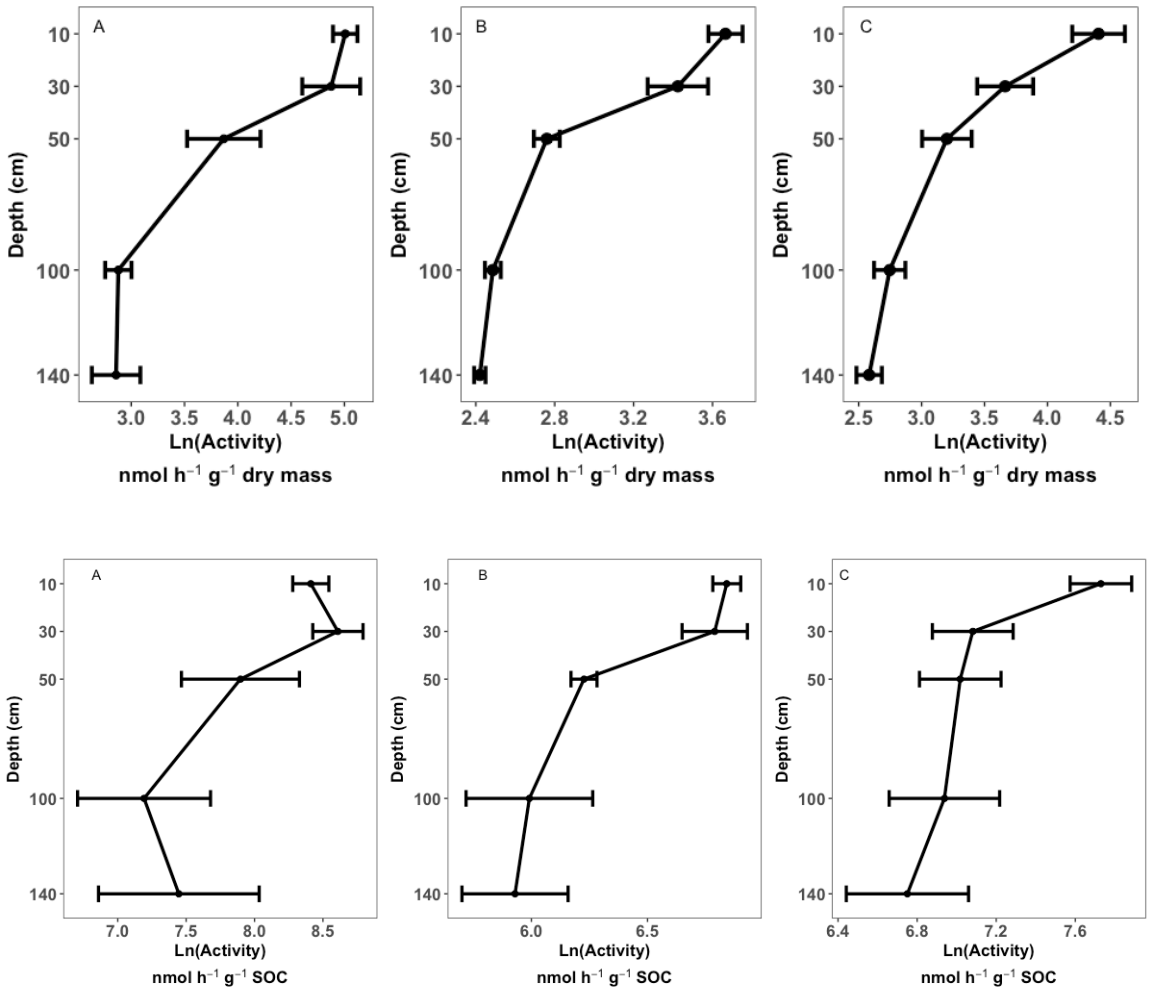

Figure 4. Top row: Decrease in average A) AP activity, B) NAG activity and C) BG activity normalized by gram of dry mass with soil depth. Values were averaged across the three trenches, for each depth.

Bottom row: Decrease in average A) AP activity, B) NAG activity and C) BG activity normalized by percent soil organic carbon with soil depth. Values were averaged across the three trenches, for each depth.

Table 5. Regression statistics relating log(EEA g dry dry) and log(EEA g SOC) to soil depth. * indicates significance at the $p<0.05$ level.

	Depth
AP (g dry mass)	0.0158*
NAG (g dry mass)	0.0319*
BG (g dry mass)	0.0128*
AP (g SOC)	0.0576
NAG (g SOC)	0.0289 *
BG (g SOC)	0.0907

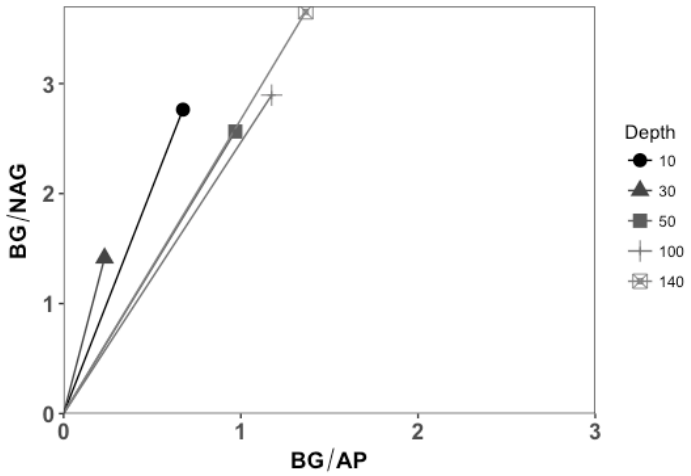

Figure 5. Ratio of BG:NAG in relation to ratio of BG:AP, for each soil depth separately. The length of the line represents more carbon limitation, while the angle of the line represents more phosphorus limitation.

Differences in the relationships between activity and depth among enzymes suggest that relative nutrient availabilities shift with depth. Vector plots indicated that soils deeper than 50cm were more carbon limited, while soils shallower than 50 cm were relatively more phosphorus limited, (Figure 5). But all vectors had slopes greater than 1.0, suggesting that P rather than N was principal constraint on microbial activity.

Enzyme activity per g dry mass was positively correlated with both percent soil organic carbon and total nitrogen (Figure 6, Table 6). The relationship between enzyme activity and total nitrogen varied depending on the enzyme: AP R^2 value: 0.77, NAG R^2 value: 0.92 and BG R^2 value: 0.81 (Figure 6, top row). We also observed differences between enzymes in the strength of the correlation between enzyme activity and percent soil organic carbon: (AP R^2 value: 0.75; NAG R^2 value: 0.90; BG R^2 value: 0.89) (Figure 6, bottom row).

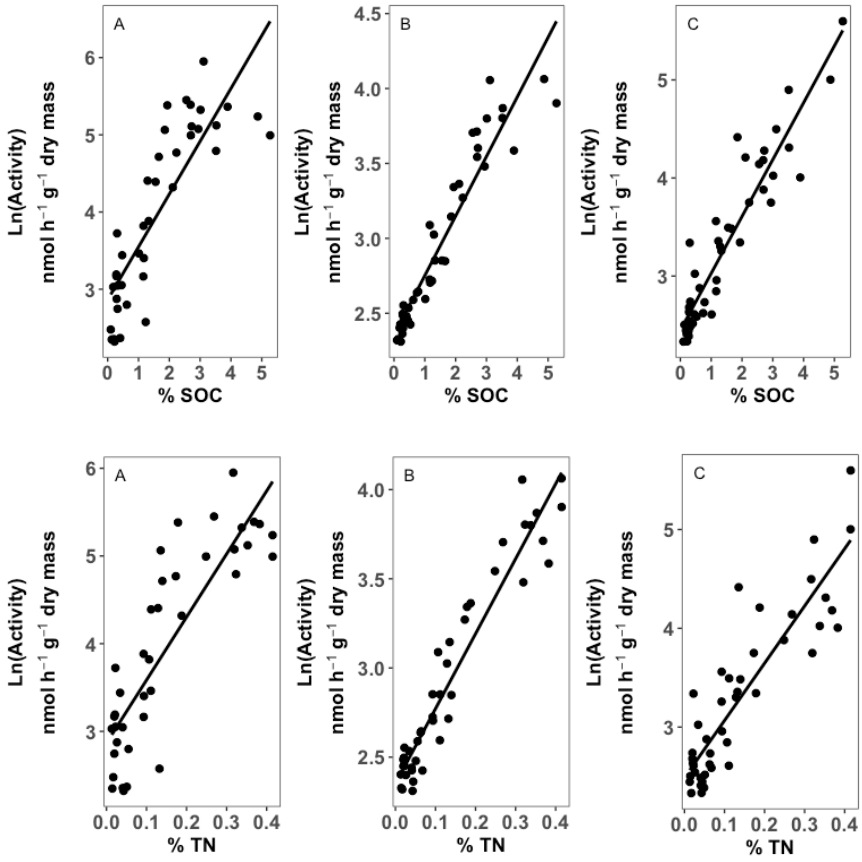

Figure 6. Top Row: Increases in A) AP activity, B) NAG activity and C) BG activity with percent soil organic carbon.

Bottom Row: Increases in A) AP activity, B) NAG activity and C) BG activity with percent total nitrogen.

Table 6. Regression statistics relating log(EEA g dry mass) to soil properties. Data is p-values for significant linear regressions.

	% SOC	% TN
AP	2.814e-13*	9.092e-14*
NAG	2.2e-16*	2.2e-16*
BG	2.2e-16*	2.2e-16*

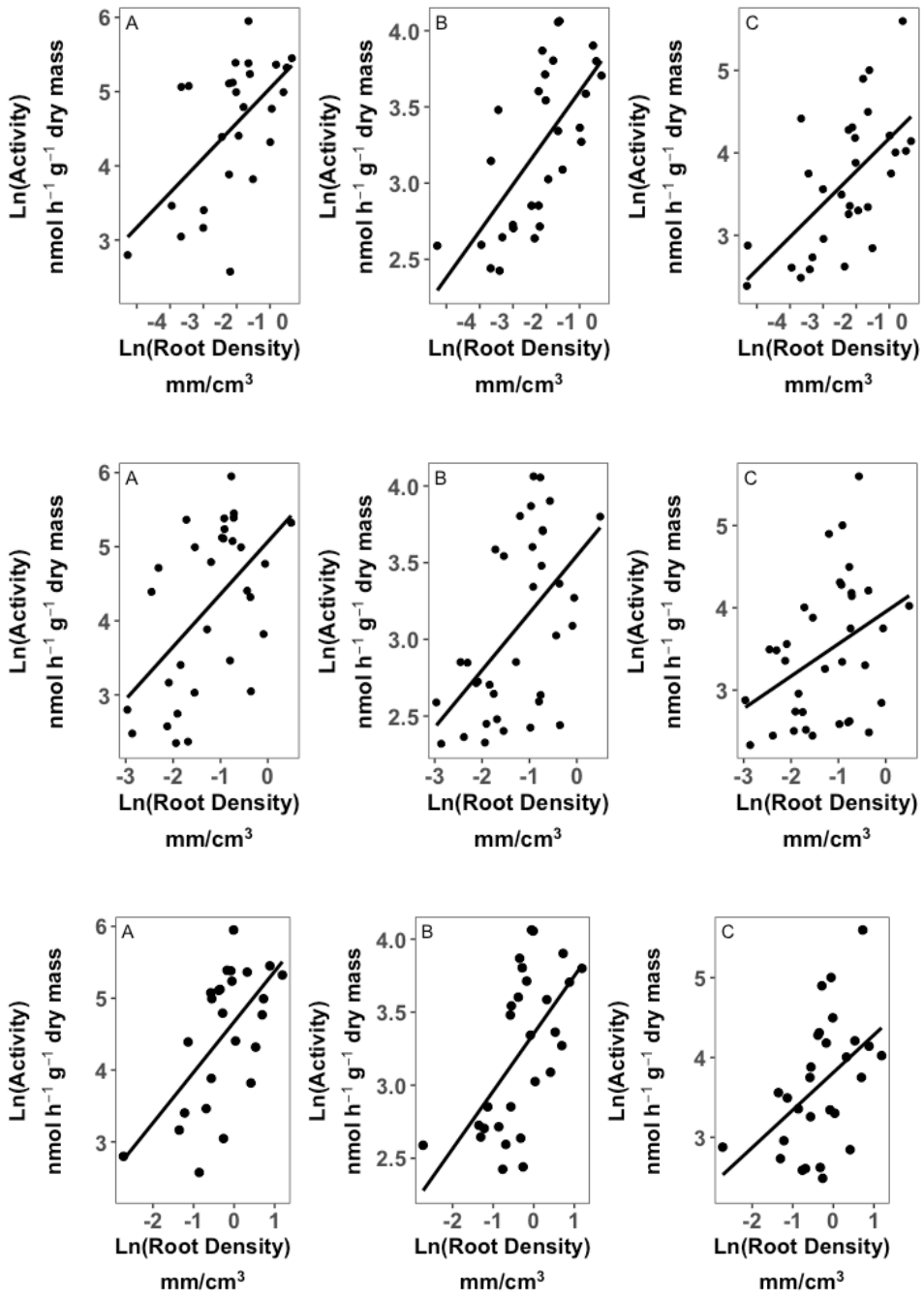

Figure 7. Increases in 1st row: A) AP activity, B) NAG activity and C) BG activity with piñon fine root density
2nd row: A) AP activity, B) NAG activity and C) BG activity with juniper fine root density
3rd row: A) AP activity, B) NAG activity and C) BG activity with total fine root density

Table 7. Regression statistics relating Ln(EEA g dry mass) to Ln(Root density) (FRD).
* indicates significance at the $p<0.05$ level

Response	**Effect**	**p-value**	**slope**
AP (g dry mass)	Piñon FRD	0.0015*	0.47
NAG (g dry mass)	Piñon FRD	0.0001*	0.31
BG (g dry mass)	Piñon FRD	0.0003*	0.40
AP (g dry mass)	Juniper FRD	0.0018*	0.71
NAG (g dry mass)	Juniper FRD	0.0009*	0.37
BG (g dry mass)	Juniper FRD	0.0225*	0.39
AP (g dry mass)	Total FRD	0.0011*	0.70
NAG (g dry mass)	Total FRD	0.0007*	0.39
BG (g dry mass)	Total FRD	0.0105*	0.47

We observed a significant, but weak relationship between AP, NAG, BG and fine root density (Figure 7, Table 7). Piñon fine root density explained 32%, 44% and 36% of the variation in AP, NAG and BG activity, respectively. Juniper fine root density explained 27%, 27% and 12% of the variation in AP, NAG and BG activity, respectively. Total fine root density explained 35%, 34% and 20% of the variation in AP, NAG and BG activity, respectively.

3.4 Bulk root enzyme activity

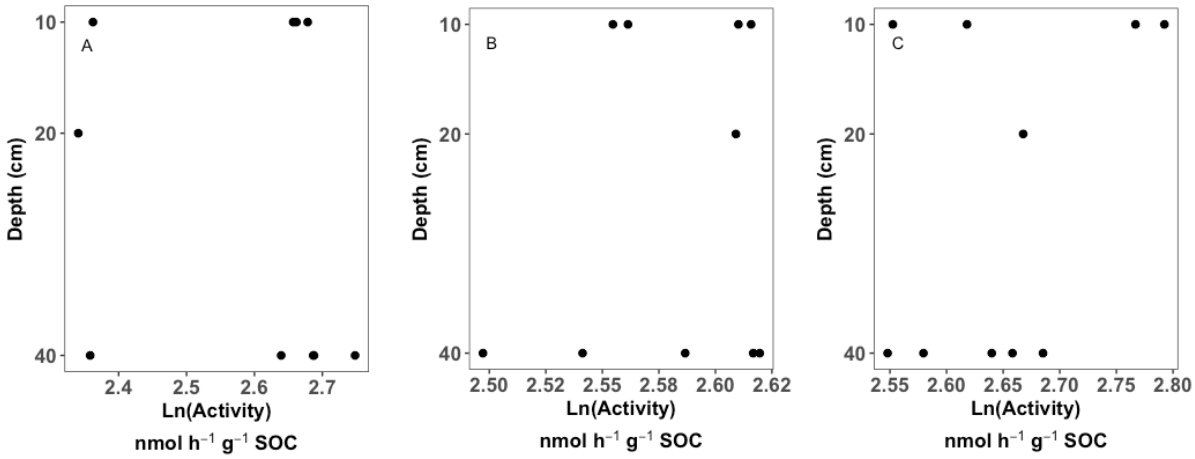

Figure 8. Root A) AP activity, B) NAG activity and C) BG activity with soil depth.

At depths 10 cm and 40 cm, soil enzyme activity per gram of SOC was higher than root enzyme activity per gram of SOC (Table 8). Root enzyme activity was not statistically correlated with soil depth (Figure 8).

Table 8. Comparison of average Ln(Soil activity per g SOC) and Ln(Root activity per g SOC)

Depth (cm)		**AP (g SOC)**	**NAG (g SOC)**	**BG (g SOC)**
10	Soil	8.41	6.84	7.73
	Root	2.59	2.59	2.68
40	Soil	8.25	6.50	7.05
	Root	2.62	2.57	2.62

3.5 Linear mixed effects model

Enzyme type, soil percent carbon and soil percent nitrogen together explained 88% of the variance in soil enzyme activity. Despite measured differences in AP, NAG and BG enzyme activity, enzyme type did not have a significant effect on enzyme activity (Table 9).

Table 9. Chi-squared values from linear-mixed effects model

Response	**Effect**	**Chi-squared**
Ln(Activity)	Enzyme type	0.5436
	Carbon	< 2.2e-16*
	Nitrogen	5.280e-12*
	Enzyme*Carbon	0.0001*
	Enzyme*Nitrogen	9.460e-05*
	Carbon*Nitrogen	1.579e-13*
	Enzyme*Carbon*Nitrogen	2.644e-05*

4. Discussion

4.1 Soil microbial enzyme activity trends with depth

To our knowledge, this is the first examination of soil microbial enzyme activity throughout the soil profile within a semi-arid ecosystem. Our results indicate that both nutrients and enzyme activity decrease with depth, which helps provide a more comprehensive understanding of microbial communities and nutrient cycling in PJ woodlands. Contrary to our first hypothesis, we did not find that canopy cover had an influence on enzyme activity. One explanation for this might be due to the lateral growth of tree roots. Krämer & Green (2000) found that acid phosphatase activity was not significantly different between soil collected underneath juniper canopies versus in the intercanopy spaces, perhaps because juniper roots extend into intercanopy spaces, and plant roots are the major producer of acid phosphatase. Enzyme activity per gram dry mass was positively correlated with both percent soil organic

carbon and total nitrogen, supporting our third hypothesis. Since PJ woodlands and other semiarid biomes are predicted to face increasing temperatures and severe drought in the coming decades (IPCC, 2007), reducing knowledge gaps about these biomes is critical in order to predict and understand future changes in these systems.

Our findings are consistent with other studies describing the relationship between belowground processes and soil depth. Taylor et al. (2002) measured microbial numbers and enzyme activity in agricultural fields in Iowa and Michigan down to 4 meters and attributed decreases in dehydrogenase, arylsulphatase and β -glucosidase activity with decreases in microbial numbers and soil organic matter. Stone et al. (2014) measured six enzymes associated with carbon, nitrogen and phosphorus acquisition and found that soil carbon, soil nitrogen and all six enzyme activities decreased exponentially with depth in Puerto Rican tropical soils. These patterns are expected because globally, soil nutrients, which influence the concentration of available substrates available for microbial communities (Sinsabaugh et al. 2009), also decrease with depth (Jobbágy & Jackson 2001). Soil carbon can also indirectly affect enzyme activities. For example, soil carbon can lead to increased moisture retention, which in turn increases substrate and enzyme diffusion (Kivlin & Treseder, 2013).

As the microbial community needs to meet their own nutrient demand via immobilization before switching to mineralization, studying enzyme activities can provide insights into elemental stoichiometry within an ecosystem (Sinsabaugh et al. 2009). The ratios of carbon:phosphorus and carbon:nitrogen acquiring enzymes (BG:AP and BG:NAG) in our soils suggest that the deeper layers at this site are more carbon limited, while the shallower layers are more phosphorus limited (Figure 5). Carbon limitation for the microbial community in the deeper layers could be due to the caliche layer, which is mainly made up of inorganic carbon and

because there are fewer roots at depth. The presence of fewer fine roots at depth is common in most forested ecosystems (López et al. 2001); fewer roots means decreases in root-derived carbon, which is a critical component of soil carbon (Allison et al. 2007). One explanation for phosphorus limitation in the shallower layers might be explained by how calcic horizons form, which involves the precipitation of elements, causing calcium-bound phosphorus to increase in calcic layers (Lajtha & Schlesinger 1988). Soils in the southwestern United States are defined by both high concentrations of precipitated carbonates, including calcium carbonate ($CaCO_3$) (Schlesinger 1982) and phosphorus limitation (Warnock et al. 2015; Sinsabaugh et al. 2008). Another explanation could be that plant-growth limiting nutrients, like phosphorus, have the shallowest distributions (Jobbágy & Jackson 2001).

Given the well- known relationship between caliche and phosphorus cycling, our finding that soils at our site are phosphorus-limited (Table 10) was not surprising and similar to the results of Warnock et al. (2015) and Sinsabaugh et al. (2008).

We unfortunately were not able to determine patterns of root enzyme activity with depth. This could be due to several factors: root enzyme assays were measured on both live and dead roots, roots were only collected down to 50 cm, as root density decreases dramatically with depth, and roots were not sorted into piñon or juniper.

Table 10. Comparison of enzyme activities in semi-arid biomes

AP (nmol h^{-1} g^{-1} SOC)	**NAG (nmol h^{-1} g^{-1} SOC)**	**BG (nmol h^{-1} g^{-1} SOC)**	**Reference**
7487	3888	533	Warnock et al. 2016
5933	4044	322	Sinsabaugh et al. 2008
4815	939	2495	Our study

4.2 Potential implications of roots at depth in this biome

Despite both a decrease in enzyme activity and nutrient concentrations throughout the soil profile, roots were found at depth. One possible explanation might be that nutrients that we did not measure are still available within deeper layers. Nutrients have different distributions within the soil profile, so although percent soil organic carbon and total nitrogen decreased with depth, studies have shown other nutrient concentrations can increase with depth. For example, Walvoord et al. 2003 found that NO_3 accumulates with depth because of evaporation and plant roots absorbing water in five arid-to-semiarid sites in the western United States.

Deeper roots within PJ woodlands might also be able to take advantage of the water holding capacity of the caliche layer. This is speculative, as this study did not explore water retention in the caliche layer. However, other studies have demonstrated a link between plant water status and caliche layer. For example, a study conducted in southern New Mexico found that creosote growing on sites with caliche had lower xylem tension than those found on sites without caliche during periods of low precipitation, as the roots were able to acquire water from the caliche (Cunningham & Burk, 1973). Water use and movement in semi-arid biomes, including PJ woodlands, is complex, owing to a variety of factors, including patchiness of vegetation (Breshears 2005), heterogeneity of soil texture and pulses in water availability (Hamerlynck et al. 2002), and the presence of a petrocalcic layer. Typically, water declines near the soil surface due to evaporation, whereas transpiration primarily drives declines in water at depth. At the same time, root density decreases with soil depth. An analysis conducted by Schenk & Jackson (2002) found that globally, on average, at least 50% of root biomass is located

in the first 30 cm of soil, but roots were found at depths 200 cm or greater in some arid and semi-arid systems. Despite water recharge events happening less frequently at depth compared to shallow layers, there is often enough water available at depth for plants to access (Chesson et al. 2004). With predictions in increased temperature, coupled with changes in the timing and size of precipitation events (IPCC, 2007), vegetation that can access water in deeper layers might be at an advantage compared to vegetation with shallower rooting strategies, thereby making them less vulnerable to drought.

5.0 Conclusions

This study demonstrated the strong link between nutrient availability and enzyme activity within a PJ woodland and showed that enzyme activity decreased with soil depth, adding vital knowledge about PJ woodlands. Similar to other studies in semi-arid biomes, AP activity was higher compared to both NAG and BG activity. PJ woodlands have experienced very high rates of mortality in recent decades and are perhaps one of the most vulnerable semi-arid biomes in the Southwestern US. These results may help increase our understanding of the important role that the caliche layer may be playing in these biomes in terms of why these biomes are so vulnerable. Future directions should include measuring the nutrient availability and water capacity of the caliche layer, and how important a barrier it plays for both nutrient and water availability in these biomes. These measurements will not only give us a more complete understanding of the relationships between above and belowground processes within this ecosystem, but also how dependent these biomes are on only the shallower pats of the soil profile.

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