Drought-induced piñon mortality alters the seasonal dynamics of microbial activity in Piñon-Juniper woodland

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Abstract

Piñon (*Pinus edulis*) - Juniper (*Juniperus monosperma*) (PJ) woodlands cover 17+ million hectares in the western USA. These woodlands have been particularly sensitive to recent changes in climate, with drought induced die-offs of piñon occurring across 1.5million ha, since 2000, triggering large changes in the structure and function of these biomes. To assess the effects of this large scale mortality on soil processes, we analyzed rhizosphere extracellular enzyme activity (EEA) and fungal biomass in soil samples collected beneath tree canopies at two piñon-juniper woodland sites: one site where piñons were experimentally killed by girdling, and a paired intact site with no piñon mortality that served as a reference. We also quantified soil water content (SWC) from soil samples, and sap flow density fluxes as an indicator of tree physiological status. Soil EEA patterns varied as functions of the identity of the closest competitor trees, host-tree physiological status, and SWC. At the girdled site, we observed higher plant cell wall related decomposition activities under live juniper canopies, compared to dead piñon trees. In contrast, at the control site, we observed higher plant cell wall decomposition rates under live piñon rather than juniper canopies, particularly with higher soil moisture availability. At the site level, ordination plots for intact PJ woodlands showed a decreasing trend in microbial cell wall decomposition activity as soil water availability, and fungal biomass increased. We observed the opposite trend at the girdled site. Overall, these results suggest that widespread piñon mortality significantly affects the functional behavior of rhizosphere microorganisms at multiple scales, in part by shifting the focal substrates of microbial community decomposition.

Key words:

piñon mortality, extracellular enzyme activity, root associated microbiota, sap flow

1. Introduction

Piñon (*Pinus edulis*) - Juniper (*Juniperus monosperma*) (PJ) woodlands cover between 17 million (Miller and Wigand 1994) and 40 million (Floyd et al., 2009; 2015) hectares in the western USA (Greenwood and Weisberg 2009). These woodlands have recently experienced multiple, prolonged, drought induced mortality of piñon trees (Breshears et al., 2009; Limousin et al., 2013; McDowell et al., 2008; Plaut et al., 2012). Estimates of total mortality are 32% to 65% for all piñons, with even greater mortality rates among the sub-population of older, reproductive piñon trees, across Colorado, New Mexico and Arizona (Floyd et al., 2009; Krofcheck et al., 2013). Given that

climate change related droughts are expected to occur more frequently in the coming decades (IPCC 2007; Liancourt et al., 2012; Smith et al., 2005), these piñon mortality events are expected to increase. Although the effects of these die-offs on above ground ecosystem processes have been the focus of multiple studies (Guardiola-Claramonte et al., 2011; Krofcheck et al., 2014; Royer et al., 2011), much less is known about how widespread piñon mortality alters soil nutrient cycles and soil microbial community composition (Berryman et al., 2013; Dean et al., in press).

Widespread tree mortality leads to increased soil organic matter (SOM), first as foliage litter deposition, then as standing dead boles, followed by deposition of coarse woody debris and eventually more SOM (Berryman et al., 2013; Ekberg et al., 2009). Initially, the microbial communities of dead piñon rhizospheres may receive pulses of labile carbon and nutrients, but the expectation is they will face a significant change in substrate quality as the host ceases to be a source of labile carbon (Berryman et al., 2013; Chen et al., 2010; Drake et al., 2013; Finér et al., 2011; Koranda et al., 2011). A shift away from labile carbon sources to more recalcitrant sources may affect soil carbon sequestration and ecosystem-atmosphere exchange rates for energy, water, and carbon, and determine how these ecosystems will respond to future climate pressures (Adams et al. 2012; Chen et al., 2010; Drake et al., 2013; Koranda et al., 2011; Plaut et al., 2012; Prescott and Grayston 2013; Yarwood et al., 2009).

The physiological responses of piñon and juniper to drought are well documented (Limousin et al 2013; McDowell et al., 2008; Plaut et al., 2012) and we predict these effects are also transmitted to their rhizosphere microbial communities. *Pinus edulis* exhibits isohydric characteristics, while *J. monosperma* exhibits anisohydric characteristics (Franks et al., 2007; Limousin et al 2013; McDowell et al., 2008; Plaut et al., 2012). Because piñon have greater stomatal control, carbon inputs to the rhizosphere are reduced when water availability decreases, while juniper are more likely to maintain physiological activity and thereby more active soil microbial communities at lower soil water contents (Bardgett et al., 2008; Berryman et al., 2013).

Because of the patchy distribution of trees within arid PJ woodland ecosystems (Coble and Hart 2013), individual rhizospheres can be considered fertility islands or hot spots for soil microbial activity (Aguilar and Sala 1999; Coble and Hart 2013). In addition, piñon and juniper support distinct root-associated microbiota (RAM) communities (Haskins and Gehring 2004; Hubert and Gehring 2008; McHugh and Gehring 2006). Within high density patches, these distinct RAM assemblages engage in direct competition, leading to reduced fungal biomass

production within the piñon RAM communities (Gehring et al., 1994; 1998, and Haskins and Gehring 2004). Based on these studies, we predict that deceased piñons will relax competition for limiting resources among their surviving, nearest-neighbors (Berryman et al., 2013; Haskins and Gehring 2004; Hubert and Gehring 2008; McHugh and Gehring 2006). This competitive release could lead to accelerated SOM turnover, increased soil respiration and faster nutrient cycling (Berryman et al., 2013), which should manifest as shifts in the magnitude and distribution of rhizosphere enzyme activity.

To assess the aggregate impacts of piñon mortality on soil microbial activity, we collected samples from an experimental PJ woodland study system in which all piñon trees larger than 7 cm diameter at breast height were girdled in 2009, and a paired control site where no trees were girdled (Krofcheck et al., 2014). We evaluated nearest neighbor and mortality effects by measuring fungal biomass and the activities of enzymes associated with nutrient acquisition and the decomposition of plant and microbial cell walls. Our hypotheses were that the rhizosphere processes of any particular grouping of living trees, at either site, would vary, with the identity and physiological status of both the RAM hosting tree, and its nearest neighbors, and that piñon mortality would alter soil processes on multiple spatial scales.

2. Materials and Methods

We conducted our study within the fetch of paired PJ woodland eddy covariance tower sites separated by 3 km, located near Mountainair, New Mexico, USA (Berryman et al., 2013; Krofcheck et al., 2014), which are part of the New Mexico Elevation Gradient (NMEG; Anderson-Teixeira et al., 2011), and the Ameriflux network. The long-term annual precipitation at the study area is 372 mm, which comes in the form of snow melt from January to March, and as seasonal, sporadic monsoonal moisture from July to October (Berryman et al., 2013; Krofcheck et al., 2014). For the year of study, 2011, winter precipitation was scarce (~20 mm) and monsoon precipitation began 4 July after an unusually dry spring. Both sites have soils characterized as lithic mollic Calciorthid (piñon channery loam), and flat terrain with less than 1% slope (see Berryman et al., 2013 for a more complete soil description). At the girdled site (34.45N, 106.21W), 1632 adult piñon trees (>7 cm diameter at breast height, DBH) were killed by girdling in September 2009, while all juvenile piñon trees and all juniper trees were left intact (Krofcheck et al., 2014). We established six replicate plots, with five nearest neighbor gradients within each plot. The gradients included a live piñon adjacent to a live piñon (LP/LP), a live piñon adjacent to a dead piñon (LP/DP), a live piñon adjacent to a live juniper (LP/LJ), a dead piñon adjacent to a live juniper (DP/LJ), and a live juniper adjacent to a

live juniper (LJ/LJ). Six similar plots were established at the untreated reference site (34.44N, 106.24W); however, at this site there were only three nearest neighbor gradients, per plot: LP/LP, LP/LJ, and LJ/LJ. The distance between canopy drip lines of the focal trees was 0.1 to 0.5m. We collected soil samples under each focal tree canopy, which we will refer to as the canopy sampling location, as well as an interspace location between the focal trees. For sampling locations located beneath similarly sized tree canopies, both living and girdled, we only collected samples from beneath standing trees. Further, when collecting the beneath tree canopy soil samples, all litter was brushed aside, to expose bare soils. Finally, we specifically avoided collecting samples covered by any understory vegetation.

2.1 Soil sampling

We collected soil samples on 6 June, 15 June, 19 July, 15 August and 28 September at the girdled site and 28 June and 15 September at the control site. At the girdled site, the LP/LP and LP/DP locations were sampled on 6 June and the LP/LJ, DP/LJ and LJ/LJ locations on 15 June. The June/ July (girdled site) and the June (control) samples represent the dry season time point before the onset of the summer monsoon, while the August/ September (girdled) and September (control) samples served as our wet season time point. For each date, we collected three 2.5 cm diameter x 10 cm deep cores, beneath each canopy and interspace location and combined them to generate a composite sample. After collection, samples were stored in an ice-filled cooler for transport to the lab. At the lab, samples were stored at 4°C until analyzed (within 72h).

2.1 Soil fungal biomass

Fungal biomass (FBM) was measured as ergosterol concentration following the protocol of Hendricks et al., (2006), and expressed as mg fungal biomass/ g soil using a conversion factor of 5.5 μg ergosterol per mg fungal biomass (Antibus and Sinsabaugh 1993, Gessner and Newell 2002).

2.2 Extracellular enzyme assays

The potential activities of alanine aminopeptidase (AAP), alkaline phosphatase (AP), β -glucosidase (BG), and β - N-acetylglucosaminidase (NAG) were measured following the protocol of Stursova et al., (2006). Activities were calculated as $\text{nmol g}^{-1} \text{h}^{-1}$.

2.3 Soil physical and chemical analyses

Gravimetric soil water content (SWC) and soil organic matter content (SOM) were determined by oven drying at 60°C for 24 h, then combusting the dried samples at 500°C for 3 h. Bulk soil pH was measured 1:1 in deionized water. Soluble PO_4^{3-} and cations (Ca^{2+} , Mg^{2+} , K^+) were extracted from 2 g of air-dried soils using 20 mL Mehlich-3 solution (Mehlich, 1984) and analyzed using a Thermal Iris Intrepid ICP-OES (Dairy One Labs, Ithaca, New York, USA). Soil mineral N availability was determined via extracting 5 g of air-dried soil with 25 mL 1M KCl. Concentrations of NO_3^- and NH_4^+ were subsequently measured with a La Chat Quick Chem 8000 flow injection analyzer (Oklahoma State University Soil, Water and Forage Analytical Laboratory, Stillwater, Oklahoma, USA). Because none of the results from these July or August girdled site samples, nor any of the results from the June control site samples showed any significant differences in soil physicochemical parameter values, we present only mean values for these samples (Table 2).

2.4 Piñon and juniper sap flow measurements:

To monitor the physiological activity of the juniper and piñon trees, we measured whole-tree sap flux using 10 mm Granier heat dissipation sap flow sensors (Granier, 1985; 1987). We installed two sensors per tree with five replicate juniper, and five replicate piñon (only small piñon, DBH < 7 cm, at the girdled site) per site. Sensors were installed at a height >1 m above ground level. We randomized installation locations with respect to tree circumference. We used two additional probes located 5 cm apart horizontally to correct for the axial temperature gradients in the stem, as specified by Goulden and Field, (1994). Sensors were covered with reflective insulation for protection. We recorded 30 min averages of the temperature difference between the heated and the unheated probes, including real-time Goulden and Field, (1994) corrections on a Campbell Scientific AM16/32 multiplexer and a CR23X data logger (Campbell Scientific). Sap flux density J_s ($\text{g} / \text{m}^2 \text{ s}$) was estimated by determining the differences between the recorded heated and unheated probe temperatures following the empirical equation of Granier (1987) and assuming zero flow during nighttime. We calculated total tree J_s by averaging the individual J_s measurements from the two sensors installed in each tree. We calculated daily means of J_s by species and reported as the mean sap flux density measured over daylight hours (PAR radiation > 15 W / m^2).

2.5 Statistical analyses

Because the experimental eddy covariance tower sites are not replicated, the focus of our analyses is the nearest neighbor effects within this patchy and seasonally dynamic landscape as it undergoes a climate driven change of state. We performed both principal components analysis (PCA), and distance based redundancy analysis (dbRDA) to assess the EEA profile for each field site, as well as data from both sites combined. Three-way nested ANOVAs, with individual sampling locations nested within sites, were conducted for each sampling date, to establish whether EEA differed across nearest neighbor gradients. Finally, when the data fulfilled assumptions of normality, we performed one-way ANOVAs to evaluate potential nearest neighbor, and seasonal rainfall effects on soil EEA patterns across the different sampling locations within each site. We made post-hoc multiple comparisons using t-tests, on all possible pairs. When normality assumptions were not met, we performed a Kruskal-Wallis non-parametric, one-way ANOVA, along with a Wilcoxon one-way test for multiple comparisons analyses. For all tests, we accepted statistical significance at an alpha of 0.05. We performed all ANOVA, and PCA analyses using JMP version 11 (SAS Institute Inc., Carey, North Carolina, USA), and dbRDA using R. For all ANOVAs, we pooled all data from both gradient end-points in the conspecific gradients, e.g., LP/LP, while data from the interspace groups, and the gradients with different end-points, e.g., DP/LJ, were all analyzed separately.

3. Results:

3.1 Multivariate analyses of all data and individual PJ woodland field sites

Multivariate analyses show clear relationships between the timing of sample collection, the specific location of sample collection, the presence of piñon mortality, the status and identity of the nearest neighbor tree and EEA rates (Table 1, Figs. 1-2). Further, both ordination methods showed a unique EEA profile for each site, with the PCA results showing these differences most clearly (Figs. 2a, 2b). The dbRDA showed that 80.7% of the variation within the data were explained by the first two components, when data from both sites were analyzed together (CAP1 54.5%, CAP2 25.7%). When considering the data from each site separately, 84.1% of the variation was explained by the first two components (axes) (CAP1 = 68.1% CAP2 = 16.0%) for the control site, and 86.5% for the girdled site (CAP1 63.3% CAP2 23.2%). Neither LP/LJ gradient showed a clear separation of points, when the location of the points are compared with those points representing the beneath canopy locations in LP/LP or LJ/LJ (Fig. 3a,3b). Finally, a PCA ordination plot of wet season girdled site data from dead piñon locations in both

LP/DP and DP/LJ, shows all points from these locations are homogeneous with respect to those representing samples collected from the living piñon and juniper rhizospheres at the same site (Fig. 4).

3.2 Extracellular enzyme activity rates:

The control site EEA rates suggest that the demands from the juniper RAM for N from peptides (AAP activity), phosphate (AP activity) and C from cellulose (BG) were greatest under junipers (LJ/LJ) in June (Fig. 5a). The June AAP rates were 170% greater for LJ/LJ, compared to LP/LP (Table s1, Fig. 5a). The mean BG activity for LJ/LJ was 411% greater than the mean activity rate for samples collected for LP/LJ (Table 2, Fig. 5a). These dry season results are indicative of soil microbial communities under juniper that were more active than piñon RAM communities.

However, by September after the onset of the summer monsoon, both AP and BG activity rates at the control site were greater (176% and 259%, respectively) in the soils collected under piñon canopies (LP/LP) compared to AP and BG activities in soils collected under juniper canopies (LJ/LJ), Table 2, Fig. 5b). Further BG activity in soils collected beneath piñon canopies (LP/LJ) were 292% greater than those collected from beneath juniper canopies (LJ/LJ), Table 2, Fig. 5b). These results from the control site suggest that the soil microbiota under piñon was more active than the juniper RAM during the wet season (Fig. 5b). More detailed data on soil EEA rates and soil physicochemical parameters are presented within Tables s1-s2 of the supplementary material.

At the girdled site, our June (dry season) results showed multiple significant differences across sampling locations, for three of four extracellular enzymes (Table 2, Fig. 6a). First, mean AAP, AP and BG activities were all significantly greater under juniper canopies (LJ/LJ, 165%, 532%, and 129%, respectively) compared to under piñon canopies (LP/LP, Table 2, Fig. 6a). For BG, activity under juniper (LJ/LJ) was 143% and 175% greater, respectively, than activity under piñon in LP/DP gradients (Tables s4, Fig. 6a). There were no significant differences between sampling locations in July as the growing season began (Table 2, Fig. 6b).

The August (wet season) results at the girdled site showed increased NAG activity under dead piñon in the DP/LJ gradient relative to all other beneath tree canopy locations, except under juniper in DP/LJ (Table 2, Fig. 7a). The September results also showed NAG activity under juniper canopies in both DP/LJ and LJ/LJ that were 236% and 121% greater than the activity rates under piñon in LP/LP (Table 2, Fig. 7b). Finally, NAG activity under dead piñon in DP/LJ was 196% greater than NAG activity under juniper in LJ/LJ, as well as being 232% and

222% higher, than either canopy location in LP/LJ (Table 2, Fig. 7b). Further, β -Gluc activity beneath juniper canopies (LJ/LJ) was 170% greater than under piñon canopies in LP/LP (Table 2, Fig. 7b). β -Gluc activity under dead piñon canopies (DP/LJ) was 159% greater compared to piñon canopies in the LP/LJ gradient. Mean AAP activity under piñon LP/LP was 181% higher than under juniper LJ/LJ (Table 2, Fig. 7b). AAP activity under piñon (LP/LP) was also 210% and 175% greater, respectively, than activity from LP/DP (Table 2, Fig. 7b). As a whole, these EEA results suggest that the soil microbial community associated with juniper rhizospheres in LJ/LJ was more active than the microbial community associated with piñon rhizospheres (Fig. 7b). The exception was AAP, which flipped from June to September, suggesting that the microbial community associated with piñon rhizospheres (LP/LP) was acquiring more N from lysed cells than the juniper associated rhizospheres in LJ/LJ (Table 2, Fig. 7b). For more detailed data on soil EEA rates and soil physicochemical parameters please refer to Tables s3-s7 of the supplementary material.

3.3. Soil fungal biomass:

Fungal biomass differed significantly across sampling gradients at both sites, but only during the wet period of the study (Fig. s1). At the control site, mean FBM under juniper in LP/LJ was 201% greater than biomass under piñon in LP/LP (Fig. s1a) ($F=2.8$, $p=0.02$). At the girdled site, FBM in August was greatest under dead piñon (Fig. s1b). In September, FBM under juniper in LJ/LJ, and DP/LJ, were 229%, and 193% greater, respectively, than FBM under live piñon in LP/LP (Fig. s1c) ($F = 2.33$, $P = 0.012$).

3.4 Piñon and juniper sap flow rates:

Sap flow density measurements from both study sites showed that during the dry, pre-monsoon conditions, April to June, day of year (DOY) 92 to 182, junipers maintained higher physiological activity than piñons (Fig. s2). This difference was more pronounced at the girdled site, where the spring mean value of daily J_s was $7.0 \text{ g m}^{-2} \text{ s}^{-1}$ in juniper and $3.4 \text{ g m}^{-2} \text{ s}^{-1}$ in piñon. At the control site, the values were $6.9 \text{ g m}^{-2} \text{ s}^{-1}$ and $5.5 \text{ g m}^{-2} \text{ s}^{-1}$ for juniper and piñon, respectively. Trees at both sites clearly increased their sap flow rates when the monsoon began, especially after a large 40 mm rain event, on August 3rd 2011 (DOY 213). However, piñon and juniper from each site, responded to wet season rainfall events differently. At the control site, juniper showed consistently greater sap flow

than piñon, throughout the period of study (Fig. s2a). J_s was $6.2 \text{ g m}^{-2} \text{ s}^{-1}$ in piñons, and $7.1 \text{ g m}^{-2} \text{ s}^{-1}$ in juniper on June 28th, and $2.6 \text{ g m}^{-2} \text{ s}^{-1}$ in piñons, vs $6.3 \text{ g m}^{-2} \text{ s}^{-1}$ in juniper on September 15th. In contrast, the remaining small piñon trees at the girdled site were much more responsive to the initial increase in soil water availability than juniper (Fig. s2b). Following the August 3rd rainfall event, the small piñon had peak flow rates reaching $22.9 \text{ g m}^{-2} \text{ s}^{-1}$ compared to $14.5 \text{ g m}^{-2} \text{ s}^{-1}$ in juniper.

3.5 Soil physicochemical properties:

Comparisons of SOM and SWC did not show any significant differences among focal groups at the control site, either across sampling locations or between wet and dry periods (Table 3). However, at the girdled site, we observed multiple significant differences in SOM and SWC suggesting that piñon mortality increased the heterogeneity of soil conditions (Table 3). Samples from the beneath juniper canopy locations in LJ/LJ had greater SOM means than those from piñon canopy samples for both June and September (Table 3). For other parameters, only September control site samples showed differences for CEC, pH, and NO_3^- (Table s7).

4. Discussion:

Our results highlight the influence of widespread piñon mortality on soil processes on both the nearest neighbor and plot scale, as a function of seasonal variation in soil moisture availability and tree physiological status (Figs. 1 and 4 - 6). These results complement findings from other studies at these sites. Berryman et al., (2013), showed that the soil microbial communities impacted by the piñon girdling, exhibited different respiration responses to seasonal increases in soil moisture and temperature. Additionally, Krofcheck et al., (2014) showed that gross primary productivity (GPP) was significantly greater at the control site, than at the girdled site. This difference in GPP between sites may contribute to the higher net ecosystem respiration rates at the girdled site (Bardgett et al., 2008; Hernandez and Hobbie 2010; Phillips et al., 2012).

4.1 Nearest neighbor species identity affects EEA profiles

Within PJ woodlands, significant neighbor effects were most pronounced for intraspecific gradients, regardless of piñon mortality, thus partially supporting our first hypothesis (Figs. 5 – 7). The PCA ordination plots show that LP/LJ data fall between the LP/LP, and LJ/LJ clusters regardless of widespread piñon mortality (Fig. 3). These results differ from those of previous studies, where strong competitive interactions were observed between

two interspecific neighbors, and their microbial associates (Haskins and Gehring, 2004a, b; Hubert and Gehring, 2008; Meinhardt and Gehring, 2012). In these studies, competitive interactions between piñon and juniper mycorrhizal communities reduced the abundance of ectomycorrhizal fungal (EMF) root tips and EMF hyphae in soils, and in some cases altered EMF community structure (Gehring et al., 1998, Haskins and Gehring 2004; Hubert and Gehring 2008).

At our PJ sites, the presence of a juniper nearest neighbor (LP/LJ) had no apparent effects on fungal biomass, nor any significant differences in EEA in the soils located beneath either piñon, or juniper canopies, at either site (Figs. 5-7, s1). Only the beneath canopy sampling locations in the LP/LP and LJ/LJ gradients showed significant differences in fungal biomass and EEA (Figs. 5-7). These functional differences are consistent with phylogenetic analyses showing that the root associated fungal communities (RAF) showed greater taxonomic richness under canopies of LP/LP and LJ/LJ pairs, compared to interspecific LP/LJ pairs (Dean et al., *accepted article*).

4.2 Piñon mortality alters soil processes at different scales

Our results show evidence for multiple, significant effects of piñon mortality on both tree physiological status, and soil microbial function. First, the ordination plot of the combined data set shows minimal overlap between sites (Fig. 2a). Second, the vectors in each plot show different associations among individual EEA rates, as well as differences in the fraction of variation explained (Figs. 2a, 2b). These responses correlate with sap flow data showing that dead piñons at the girdled site altered the physiological activity of the surviving trees (Fig. s2b). Collectively, these changes underlie the stand level R_s/R_{ES} , and GPP responses, reported by Berryman et al., (2013) and Krofcheck et al., (2014).

The wet season results from the DP/LJ and LP/DP gradient also highlight the interaction between piñon mortality, and seasonal monsoonal rainfall. Points representing the BG activities from samples collected beneath dead piñons were more similar to those from living rhizospheres, than they were to being displayed as discrete groups representing RAM activities within dead piñon rhizospheres (Fig. 4). Further, soil samples collected beneath living piñon tree canopies (LP/LP) in September had high AAP rates and low BG rates in contrast to soil collected beneath dead piñons in (DP/LJ) (Fig. 7). These contrasts between dead vs. living rhizospheres, are similar to those

reported previously (Chen et al., 2010; Drigo et al., 2012; Koranda et al., 2011; Yarwood et al., 2009). Collectively, these studies provide evidence of shifts from soil fungal communities dominated by biotrophic mycorrhizal fungi, to communities dominated by saprobic organisms. Drigo et al., (2012) suggested that once dead EM fungal hyphae are present, saprotrophic fungi can metabolize the necromass, using NAG. We observed the greatest NAG activity under dead piñons, throughout the wet season (Fig. 7), which fits with the results of Drigo et al., (2012).

4.3 Differences in tree physiology, soil moisture, and piñon mortality affect soil microbial community functional behaviors

Given that juniper are anisohydric, and piñon are isohydric, it is not surprising that dry season EEA and J_s was greater under juniper. During the monsoon period, tree activity was significantly greater (Fig. s2), leading to greater EEA rates under piñon (LP/LP) than juniper (LJ/LJ) (Fig. 7b). More interesting, the surviving isohydric piñon and their RAM at the girdled site were more responsive to increases in SWC brought about by precipitation pulses, yielding higher sap flow and AAP activity (Figs. s2b, and 7b). In previous studies, increased piñon sap flow lead to increased photosynthetic activity, which may prioritize N acquisition, to support RuBisCO, and needle production (Lajtha and Getz 1993; Lambers et al., 2008: and West et al., 2007; 2008). The large increases in soil AAP, relative to other activities, are consistent with greater demand and perhaps competition for N between plants and microbes.

5. Conclusions:

Overall, our results supported our hypotheses that nearest neighbor identity and status, e.g., living or dead, lead to differences in EEA rates. At the ecosystem scale, piñon mortality may also amplify these trends, in part, by affecting shallow SWC availability e.g., in the top five to 10 cm (Berryman et al., 2013). In conclusion, our results show the importance of including multiple time points in experiments, i.e. a simulated drought induced piñon die-off, to address the consequences of global change. If we had not sampled over a four-month period, we would not have seen, for example, how quickly and dramatically a piñon associated microbial community can accelerate their peptidase activity in response to changes in soil conditions and tree activity. Finally, based on these results we are currently witnessing a general shift in soil microbial community activity profiles as a response to drought-induced tree mortality.

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Figure Legends

Figure 1. The ordination plot from the PCA analyses of all data considered together.

Figure 2. Graphical results from PCA analyses of A) all data from the control site, and B) all data from the girdled site.

Figure 3. PCA ordination plots from the LP/LP, LJ/LJ, and LP/LJ gradients from the a) control site data, and b) the girdled site data. For both plots, the sampling location number corresponds to the following beneath tree canopy locations, one (LP) in LP/LP, six (LP) in LP/LJ, eight (LJ) in LP/LJ, and twelve (LJ) in LJ/LJ.

Figure 4. A PCA ordination plot of the August and September FBM, β -Gluc, and NAGase data from the LP/LP, LP/DP, LP/LJ, DP/LJ, and LJ/LJ gradients at the girdled site. The sampling location number provided in the figure legend, corresponds to the following beneath tree canopy locations, one (LP) in LP/LP, three (LP) in LP/DP, five (DP) in LP/DP, six (LP) LP/LJ, eight (LJ) in LP/LJ, nine (DP) in DP/LJ, eleven (LJ) in DP/LJ, and twelve (LJ) in LJ/LJ.

Figure 5. Mean soil enzyme activity rates from the control site, for both A) dry period 2011 and B) wet period 2011. Y axis scale is the same, for both panels.

Figure 6. Mean soil enzyme activity rates for A) June and B) July 2011, i.e., the dry period, from the girdled site. Y axis scale is the same, for both panels.

Figure 7. Mean soil enzyme activity rates for A) August and B) September 2011, i.e the wet period, from the girdle site. Y axis scale is the same for both panels.

Table 1. ANOVA results from three-way nested ANOVA analyses of data for each hydrolytic EEA rate, which compared means from sampling locations and sampling times that were common to both study sites.

Response	Effect	Degrees of Freedom	Sum of Squares	F value	p value
Fungal Biomass	Sampling Day	2	5.81468	5.147	0.0066
	Site	1	2.240125	3.966	0.0478
	Sampling Location[Site]	12	20.154489	2.974	0.0008
Peptidase	Sampling Day	2	2027793081	70.344	<.0001
	Site	1	3721620891	258.206	<.0001
	Sampling Location[Site]	12	224502100	1.298	0.2218
Phosphatase	Sampling Day	2	211810419	2.833	0.0612
	Site	1	576352877	15.418	0.0001
	Sampling Location[Site]	12	537678464	1.199	0.2861
β-Gluc	Sampling Day	2	54532178	63.806	<.0001
	Site	1	3949485	9.242	0.0027
	Sampling Location[Site]	12	9292253	1.812	0.0482
NAGase	Sampling Day	2	73436258	9.912	<.0001
	Site	1	102456515	27.657	<.0001
	Sampling Location[Site]	12	53030782	1.193	0.2901

Table 2. One-way ANOVA statistics from the EEA results from all individual sampling locations, from both sites, and for all sampling dates.

Site	Sampling period	¹ AlaAP (nmol/h/g OM)	¹ Alk P (nmol/h/g OM)	β-Gluc (nmol/h/g OM)	NAG (nmol/h/g OM)
control	June	K=15.0 P=0.02	No data	K=8.7 P=0.19	F=1.88 P=0.105
control	September	F=0.78 P=0.57	F=2.42 P=0.041	K=14.5 P=0.025	F=2.16 P=0.63
girdled	June	K=45.4 P<0.0001	K=38.8 P<0.0001	F=3.04 P=0.0016	K=27.2 P=0.0072
girdled	July	K=2.64 P=.099	F=0.51 P=0.724	K=15.1 P=0.24	K=14.8 P=0.25
girdled	August	K=45.4 P<0.0001	K=38.8 P<0.0001	F=3.04 P=0.0016	K=27.2 P=0.0072
girdled	September	K=45.4 P<0.0001	K=38.8 P<0.0001	F=3.04 P=0.0016	K=27.2 P=0.0072

Table 3. Soil water and soil organic matter contents for both sites, from all sampling dates. Values in table correspond to values included in calculations for soil EEA's shown in Figures 3-5.

Site	Sampling Gradient	Sampling Location	June 2011		August 2011		September 2011	
			SWC ± (1 SD)	gOM/ g Soil	SWC ± (1 SD)	gOM/ g Soil	SWC ± (1 SD)	gOM/ g Soil
control	All averaged together	Site-wide means	0.034 (0.01)	0.089 (0.027)	No Data	No Data	0.11 (0.02)	0.12 (0.044)
girdled	(LJ/LJ)	live juniper	0.051 (0.007) ^{BCD}	0.11 (0.033) ^A	16.678 (1.475) ^{AB}	0.10 (0.024)	8.90 (1.74) ^A	0.14 (0.033) ^A
girdled	(LP/LJ)	live juniper	0.039 (0.008) ^{DE}	0.093 (0.017) ^{ABC}	15.154 (2.325) ^{ABC}	0.13 (0.039)	7.50 (1.50) ^{BC}	0.10 (0.028) ^{BCD}
girdled	(DP/LJ)	live juniper	0.046 (0.016) ^{CDE}	0.096 (0.030) ^{AB}	14.188 (1.090) ^D	0.12 (0.029)	6.75 (0.52) ^C	0.13 (0.050) ^{AB}
girdled	(LP/LP)	live piñons	0.057 (0.005) ^{BC}	0.080 (0.014) ^{BC}	17.292 (1.790) ^A	0.13 (0.042)	6.74 (0.81) ^C	0.09 (0.017) ^D
girdled	(LP/DP)	live piñon	0.073 (0.017) ^A	0.083 (0.016) ^{ABC}	14.58 (1.136) ^{BCD}	0.15 (0.014)	6.90 (2.19) ^{BC}	0.12 (0.034) ^{BCD}
girdled	(LP/DP)	dead piñon	0.060 (0.019) ^{ABC}	0.096 (0.022) ^{AB}	14.077 (1.295) ^{CD}	0.12 (0.029)	6.14 (0.72) ^C	0.11 (0.018) ^{ABC}
girdled	(LP/LJ)	live piñon	0.043 (0.011) ^{DE}	0.11 (0.035) ^A	18.047 (1.739) ^A	0.12 (0.023)	7.26 (0.92) ^{BC}	0.09 (0.022) ^{CD}
girdled	(DP/LJ)	dead piñon	0.0380 (0.010) ^E	0.096 (0.032) ^{AB}	14.369 (2.662) ^{BCD}	0.11 (0.021)	6.74 (0.64) ^C	0.12 (0.041) ^{BCD}
			F = 4.93 P < 0.0001	F = 2.54 P = 0.028	F=4.06 P=0.0015	F=1.55 P=0.17	K=21.8 P=0.04	F=3.77 P=0.003